STIC Search Report
Biotech-Chem Library

STIC Database Tracking Number: 173118

TO: Satayanarayana Gudibande Location: REM/3C04/3C18 Art Unit: 1654 Friday, December 09, 2005 Case Serial Number: 10/500302	From: Mary Hale Location: Biotech/Chem Library Rem 1D86 Phone: 2-2507 Mary.Hale@uspto.gov

Search Notes

Feel free to contact me if you have any questions.

Note -- results are printed on both sides of printout

Dudibande
10/500302


```
=> fil reg
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.42       0.42

FILE 'REGISTRY' ENTERED AT 15:36:15 ON 09 DEC 2005
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STRUCTURE FILE UPDATES:    8 DEC 2005  HIGHEST RN 869627-02-1
DICTIONARY FILE UPDATES:   8 DEC 2005  HIGHEST RN 869627-02-1

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TSCA INFORMATION NOW CURRENT THROUGH JULY 14, 2005

  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.

**********************************************************************
*                                                                    *
* The CA roles and document type information have been removed from *
* the IDE default display format and the ED field has been added,   *
* effective March 20, 2005.  A new display format, IDERL, is now    *
* available and contains the CA role and document type information. *
*                                                                    *
**********************************************************************

Structure search iteration limits have been increased.  See HELP SLIMITS
for details.

REGISTRY includes numerically searchable data for experimental and
predicted properties as well as tags indicating availability of
experimental property data in the original document.  For information
on property searching in REGISTRY, refer to:

http://www.cas.org/ONLINE/UG/regprops.html

=> e nonapeptide/cn 5
E1            1     NONAPENTAERYTHRITOL EICOSABEHENATE/CN
E2            1     NONAPENTAFULVALENE/CN
E3            0 --> NONAPEPTIDE/CN
E4            1     NONAPEPTIDE FUSION PROTEIN WITH 2-104-RIBONUCLEASE (RANA PIP
                    IENS)/CN
E5            1     NONAPERDEUTEROMETHYL-6,8-DI-C-RHAMNOSYLAPIGENIN/CN

=> e decapeptide/cn 5
E1            1     DECAPENTAYNE, DI-9-PHENANTHRYL-/CN
E2            1     DECAPENTAYNE, DIPHENYL-/CN
E3            0 --> DECAPEPTIDE/CN
E4            1     DECAPEPTIDE I/CN
E5            1     DECAPEPTYL/CN

=> s (methanol or ethanol or propanol or isopropanol)/cn
              1 METHANOL/CN
              1 ETHANOL/CN
```

```
             2 PROPANOL/CN
             1 ISOPROPANOL/CN
L1           5 (METHANOL OR ETHANOL OR PROPANOL OR ISOPROPANOL)/CN

=> s (methyl acetate or ethyl acetate or methyl propionate or ethyl propionate or
ethyl isopropionate or butyl acetate or isobutyl acetate or "t-butyl acetate" or
ethyl formate or propyl formate or isopropyl formate or hexane or heptane or octane
or cyclohexane or methylcyclohexane)/cn
             1 METHYL ACETATE/CN
             1 ETHYL ACETATE/CN
             1 METHYL PROPIONATE/CN
             1 ETHYL PROPIONATE/CN
             0 ETHYL ISOPROPIONATE/CN
             1 BUTYL ACETATE/CN
             1 ISOBUTYL ACETATE/CN
             0 "T-BUTYL ACETATE"/CN
             1 ETHYL FORMATE/CN
             1 PROPYL FORMATE/CN
             1 ISOPROPYL FORMATE/CN
             1 HEXANE/CN
             1 HEPTANE/CN
             1 OCTANE/CN
             1 CYCLOHEXANE/CN
             1 METHYLCYCLOHEXANE/CN
L2          14 (METHYL ACETATE OR ETHYL ACETATE OR METHYL PROPIONATE OR ETHYL
               PROPIONATE OR ETHYL ISOPROPIONATE OR BUTYL ACETATE OR ISOBUTYL
               ACETATE OR "T-BUTYL ACETATE" OR ETHYL FORMATE OR PROPYL FORMATE
               OR ISOPROPYL FORMATE OR HEXANE OR HEPTANE OR OCTANE OR CYCLOHEXA
               NE OR METHYLCYCLOHEXANE)/CN

=> s (".tertiary.- butyl acetate" or acetic acid or propionic acid)/cn
             0 ".TERTIARY.- BUTYL ACETATE"/CN
             1 ACETIC ACID/CN
             1 PROPIONIC ACID/CN
L3           2 (".TERTIARY.- BUTYL ACETATE" OR ACETIC ACID OR PROPIONIC ACID)/C
               N

=> fil medl,biosis,embase,caplus
COST IN U.S. DOLLARS                          SINCE FILE      TOTAL
                                                 ENTRY    SESSION
FULL ESTIMATED COST                              108.38     108.80

FILE 'MEDLINE' ENTERED AT 15:39:41 ON 09 DEC 2005

FILE 'BIOSIS' ENTERED AT 15:39:41 ON 09 DEC 2005
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FILE 'CAPLUS' ENTERED AT 15:39:41 ON 09 DEC 2005
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=> s l2 or l3 or methyl acetate or ethyl acetate or methyl propionate or ethyl
propionate or ethyl isopropionate or butyl acetate or isobutyl acetate or "t-butyl
acetate" or ethyl formate or propyl formate or isopropyl formate or hexane or
heptane or octane or cyclohexane or methylcyclohexane
L4       23205 FILE MEDLINE
```

Prepared by: Mary Hale @2-2507 Rem Bldg 1D86

```
L5        34113 FILE BIOSIS
L6        39078 FILE EMBASE
L7       417772 FILE CAPLUS

TOTAL FOR ALL FILES
L8       514168 L2 OR L3 OR METHYL ACETATE OR ETHYL ACETATE OR METHYL PROPIONATE
                 OR ETHYL PROPIONATE OR ETHYL ISOPROPIONATE OR BUTYL ACETATE OR
                ISOBUTYL ACETATE OR "T-BUTYL ACETATE" OR ETHYL FORMATE OR PROPYL
                 FORMATE OR ISOPROPYL FORMATE OR HEXANE OR HEPTANE OR OCTANE OR
                CYCLOHEXANE OR METHYLCYCLOHEXANE

=> s acetic acid or propionic acid
L9        33366 FILE MEDLINE
L10       46001 FILE BIOSIS
L11       44680 FILE EMBASE
L12      223501 FILE CAPLUS

TOTAL FOR ALL FILES
L13      347548 ACETIC ACID OR PROPIONIC ACID

=> s l8 or l13
L14       50602 FILE MEDLINE
L15       69093 FILE BIOSIS
L16       64225 FILE EMBASE
L17      544494 FILE CAPLUS

TOTAL FOR ALL FILES
L18      728414 L8 OR L13

=> s l1 or methanol or ethanol or propanol or isopropanol
L19      103185 FILE MEDLINE
L20      172173 FILE BIOSIS
L21      141509 FILE EMBASE
L22      506717 FILE CAPLUS

TOTAL FOR ALL FILES
L23      923584 L1 OR METHANOL OR ETHANOL OR PROPANOL OR ISOPROPANOL


=> fil medl,biosis,embase,caplus
COST IN U.S. DOLLARS                        SINCE FILE      TOTAL
                                               ENTRY     SESSION
FULL ESTIMATED COST                             0.45      181.55

FILE 'MEDLINE' ENTERED AT 15:42:30 ON 09 DEC 2005

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=> s nonapeptide? or decapeptide?
L24        3301 FILE MEDLINE
L25        3454 FILE BIOSIS


Prepared by: Mary Hale @2-2507 Rem Bldg 1D86
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L26        3129 FILE EMBASE
L27        5580 FILE CAPLUS

TOTAL FOR ALL FILES
L28       15464 NONAPEPTIDE? OR DECAPEPTIDE?

=> s l28 and (dissol? solvent? or (water and l23))
L29          11 FILE MEDLINE
L30          17 FILE BIOSIS
L31           9 FILE EMBASE
L32          17 FILE CAPLUS

TOTAL FOR ALL FILES
L33          54 L28 AND (DISSOL? SOLVENT? OR (WATER AND L23))

=> s l33 and l18
L34           3 FILE MEDLINE
L35           5 FILE BIOSIS
L36           3 FILE EMBASE
L37           5 FILE CAPLUS

TOTAL FOR ALL FILES
L38          16 L33 AND L18

=> dup rem l38
PROCESSING COMPLETED FOR L38
L39           8 DUP REM L38 (8 DUPLICATES REMOVED)

=> d 1-8 ibib abs hit

L39  ANSWER 1 OF 8     MEDLINE on STN                    DUPLICATE 1
ACCESSION NUMBER:   2004056321     MEDLINE
DOCUMENT NUMBER:    PubMed ID: 14757490
TITLE:              Adsorption of the decapeptide Cetrorelix depends
                    both on the composition of dissolution medium and the type
                    of solid surface.
AUTHOR:             Grohganz Holger; Rischer Matthias; Brandl Martin
CORPORATE SOURCE:   Department of Pharmaceutics and Biopharmaceutics, Institute
                    of Pharmacy, University of Tromso, N-9037, Tromso, Norway..
                    holgerg@farmasi.uit.no
SOURCE:             European journal of pharmaceutical sciences : official
                    journal of the European Federation for Pharmaceutical
                    Sciences, (2004 Feb) 21 (2-3) 191-6.
                    Journal code: 9317982. ISSN: 0928-0987.
PUB. COUNTRY:       Netherlands
DOCUMENT TYPE:      Journal; Article; (JOURNAL ARTICLE)
LANGUAGE:           English
FILE SEGMENT:       Priority Journals
ENTRY MONTH:        200411
ENTRY DATE:         Entered STN: 20040204
                    Last Updated on STN: 20041219
                    Entered Medline: 20041126
AB   High performance liquid chromatography (HPLC) analysis of increasing
     amounts of the decapeptide Cetrorelix, a potent antagonist of
     the luteinising hormone-releasing hormone, in distilled water
     resulted in a poor and variable response when solutions of low
     concentration (0.2-4microg/ml) were analysed.  Rinsing experiments
     revealed loss of analyte due to adsorption to the vial surfaces as the
     main reason for this.  The adsorption of Cetrorelix was found to follow a
     Langmuir isotherm reaching a plateau at 0.4microg/cm(2) and to be
```

influenced by both the dissolution medium and the type of vial used. The adsorption tendency of Cetrorelix was reduced by: (a) a more lipophilic solvent (**ethanol**), (b) a more acidic pH (**acetic acid**) inducing repulsive charges (c) a micellar solution of various tensides. With all of these media the HPLC response was higher (up to five times) and less variable. Adsorption of Cetrorelix to solid surfaces decreased in the rank order: glass > polypropylene = polyethylene > poly-(tetrafluoroethylene), with considerable differences between the glass vials of various suppliers.

TI Adsorption of the **decapeptide** Cetrorelix depends both on the composition of dissolution medium and the type of solid surface.

AB High performance liquid chromatography (HPLC) analysis of increasing amounts of the **decapeptide** Cetrorelix, a potent antagonist of the luteinising hormone-releasing hormone, in distilled **water** resulted in a poor and variable response when solutions of low concentration (0.2-4microg/ml) were analysed. Rinsing experiments revealed loss of analyte due to adsorption to the vial surfaces as the main reason for this. The adsorption of Cetrorelix was found to follow a Langmuir isotherm reaching a plateau at 0.4microg/cm(2) and to be influenced by both the dissolution medium and the type of vial used. The adsorption tendency of Cetrorelix was reduced by: (a) a more lipophilic solvent (**ethanol**), (b) a more acidic pH (**acetic acid**) inducing repulsive charges (c) a micellar solution of various tensides. With all of these media the HPLC response was higher (up to five times) and less variable. Adsorption of Cetrorelix to solid surfaces decreased in the rank order: glass > polypropylene = polyethylene > poly-(tetrafluoroethylene), with considerable differences between the glass vials of various suppliers.

CT Check Tags: Comparative Study
Acetic Acid: CH, chemistry
Adsorption
Chromatography, High Pressure Liquid
Drug Packaging
Ethanol: CH, chemistry
Glass: CH, chemistry
*Gonadorelin: AA, analogs & derivatives
*Gonadorelin: AI, antagonists & inhibitors
*Gonadorelin: CH, chemistry
Hydrogen-Ion Concentration
Octoxynol: CH, chemistry
Polypropylenes: CH, chemistry
Polytetrafluoroethylene: CH, chemistry
Research Support, Non-U.S. Gov't
Solubility
*Solvents: CH, chemistry
Surface Properties
Water: CH, chemistry

RN 120287-85-6 (cetrorelix); 33515-09-2 (Gonadorelin); **64-17-5 (Ethanol); 64-19-7 (Acetic Acid); 7732-18-5 (Water)** ; 9002-84-0 (Polytetrafluoroethylene); 9002-93-1 (Octoxynol)

L39 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2003:532678 CAPLUS
DOCUMENT NUMBER: 139:53318
TITLE: Peptide purification
INVENTOR(S): Rasmussen, Jon H.; Rasmussen, Palle H.
PATENT ASSIGNEE(S): Polypeptide Laboratories A/S, Den.
SOURCE: PCT Int. Appl., 13 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent

Prepared by: Mary Hale @2-2507 Rem Bldg 1D86

LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2003055900	A1	20030710	WO 2002-IB5581	20021223
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
CA 2471717	AA	20030710	CA 2002-2471717	20021223
EP 1468009	A1	20041020	EP 2002-783479	20021223
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, SK				
JP 2005515217	T2	20050526	JP 2003-556430	20021223
ZA 2004005135	A	20050621	ZA 2004-5135	20040628
NO 2004003046	A	20040830	NO 2004-3046	20040716
PRIORITY APPLN. INFO.:			SE 2001-4462 A	20011229
			WO 2002-IB5581 W	20021223

AB A nona- or **decapeptide** is purified from residual organic solvent by dissolving in a solvent comprising water and at least one C1-C3 alc. followed by precipitation into a vigorously stirred solvent consisting of an alkyl ester of a carboxylic acid (3 to 6 carbon atoms) and one or several non-polar compds. (**hexane**, **heptane**, **octane**, **cyclohexane**, or **methylcyclohexane**) and optionally up to 5 % acetic or **propionic acid**, isolating the precipitated nona- or **decapeptide**, followed by washing with a mixture of C3-C5 esters and drying [with the proviso that the water content of the solvent comprising water and the at least one alc. is below 8 % (volume/volume) and the volume ratio of the **dissoln. solvent** mixture and the precipitation solvent mixture is 1:10 or higher]. The procedure was applied to Ac-D-2Nal-D-4ClPhe-D-3Pal-Ser-MeTyr-D-Asn-Leu-Lys(iPr)-Pro-D-Ala-NH2 [2Nal = 3-(2-naphthyl)alanine; 4-ClPhe = 4-chlorophenylalanine; 3Pal = 3-(3-pyridyl)alanine], which was obtained as the monoacetate in 99.8% HPLC purity.

REFERENCE COUNT: 5 THERE ARE 5 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

AB A nona- or **decapeptide** is purified from residual organic solvent by dissolving in a solvent comprising water and at least one C1-C3 alc. followed by precipitation into a vigorously stirred solvent consisting of an alkyl ester of a carboxylic acid (3 to 6 carbon atoms) and one or several non-polar compds. (**hexane**, **heptane**, **octane**, **cyclohexane**, or **methylcyclohexane**) and optionally up to 5 % acetic or **propionic acid**, isolating the precipitated nona- or **decapeptide**, followed by washing with a mixture of C3-C5 esters and drying [with the proviso that the water content of the solvent comprising water and the at least one alc. is below 8 % (volume/volume) and the volume ratio of the **dissoln. solvent** mixture and the precipitation solvent mixture is 1:10 or higher]. The procedure was applied to Ac-D-2Nal-D-4ClPhe-D-3Pal-Ser-MeTyr-D-Asn-Leu-Lys(iPr)-Pro-D-Ala-NH2 [2Nal = 3-(2-naphthyl)alanine; 4-ClPhe = 4-chlorophenylalanine; 3Pal =

3-(3-pyridyl)alanine], which was obtained as the monoacetate in 99.8% HPLC purity.

IT Peptides, preparation
RL: PUR (Purification or recovery); PREP (Preparation)
(purification of nona- or **decapeptides**)

IT 183552-38-7P 547741-72-0P
RL: PUR (Purification or recovery); PREP (Preparation)
(purification of nona- or **decapeptides**)

L39 ANSWER 3 OF 8 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2002671687 MEDLINE
DOCUMENT NUMBER: PubMed ID: 12431727
TITLE: Four novel PYFs: members of NPY/PP peptide superfamily from the eyestalk of the giant tiger prawn Penaeus monodon.
AUTHOR: Sithigorngul Paisarn; Pupuem Jirasak; Krungkasem Chatchadaporn; Longyant Siwaporn; Panchan Nanthika; Chaivisuthangkura Parin; Sithigorngul Weerawan; Petsom Amorn
CORPORATE SOURCE: Department of Biology, Faculty of Science, Srinakharinwirot University, Sukhumvit 23, Bangkok 10110, Thailand.. paisarn@swu.ac.th
SOURCE: Peptides, (2002 Nov) 23 (11) 1895-906.
Journal code: 8008690. ISSN: 0196-9781.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200306
ENTRY DATE: Entered STN: 20021115
Last Updated on STN: 20030613
Entered Medline: 20030612

AB An immunocytochemical method was used for localization of pancreatic polypeptide (PP) immunoreactive substances in the eyestalk of Penaeus monodon using anti-C-terminal hexapeptide of PP (anti-PP6) antiserum. Approximately 200 neuronal cell bodies were recognized in the ganglia between the medulla interna (MI) and medulla terminalis (MT) and surrounding MT in conjunction with the neuronal processes in medulla externa (ME), MI, MT and sinus gland. About half of the PP immunoreactive neurons were also recognized by a combination of three monoclonal antibodies raised against FMRFamide-like peptides. Isolation of the PP immunoreactive substances from the eyestalk was performed using 7500 eyestalks extracted in **methanol/acetic acid/ water** (90/1/9) followed by five to six steps of RP-HPLC separation. Dot-ELISA with anti-PP6 antiserum was used to monitor PP-like substances in various fractions during the purification processes. Four new sequences of one hexapeptide; RARPRFamide, and three **nonapeptides**; YSQVSRPRFamide, YAIAGRPRFamide and YSLRARPRFamide were identified, and named as Pem-PYF1-4 due to their structural similarity to the PYF found in squid Loligo vulgaris. Each of the new peptides shares four to seven common residues with the C-terminus of the squid PYF and with the NPFs found in other invertebrates. The NPY/PP superfamily as well as the FMRFamide peptide family may be present throughout vertebrates and invertebrates.

AB An immunocytochemical method was used for localization of pancreatic polypeptide (PP) immunoreactive substances in the eyestalk of Penaeus monodon using anti-C-terminal hexapeptide of PP (anti-PP6) antiserum. Approximately 200 neuronal cell bodies were recognized in the ganglia between the medulla interna (MI) and medulla terminalis (MT) and surrounding MT in conjunction with the neuronal processes in medulla externa (ME), MI, MT and sinus gland. About half of the PP immunoreactive

neurons were also recognized by a combination of three monoclonal antibodies raised against FMRFamide-like peptides. Isolation of the PP immunoreactive substances from the eyestalk was performed using 7500 eyestalks extracted in **methanol/acetic acid/ water** (90/1/9) followed by five to six steps of RP-HPLC separation. Dot-ELISA with anti-PP6 antiserum was used to monitor PP-like substances in various fractions during the purification processes. Four new sequences of one hexapeptide; RARPRFamide, and three **nonapeptides**; YSQVSRPRFamide, YAIAGRPRFamide and YSLRARPRFamide were identified, and named as Pem-PYF1-4 due to their structural similarity to the PYF found in squid Loligo vulgaris. Each of the new peptides shares four to seven common residues with the C-terminus of the squid PYF and with the NPFs found in other invertebrates. The NPY/PP superfamily as well as the FMRFamide peptide family may be present throughout vertebrates and invertebrates.

L39 ANSWER 4 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
DUPLICATE 3

ACCESSION NUMBER: 1996:160876 BIOSIS
DOCUMENT NUMBER: PREV199698733011
TITLE: Purification of synthetic lipopeptide conjugates by liquid chromatography.
AUTHOR(S): Winger, T. M.; Ludovice, P. J.; Chaikof, E. L.
CORPORATE SOURCE: Sch. Chemical Engineering, Ga. Inst. Technology, Atlanta, GA 30332, USA
SOURCE: Journal of Liquid Chromatography, (1995) Vol. 18, No. 20, pp. 4117-4125.
CODEN: JLCHD8. ISSN: 0148-3919.
DOCUMENT TYPE: Article
LANGUAGE: English
ENTRY DATE: Entered STN: 11 Apr 1996
Last Updated on STN: 2 May 1996

AB Conventional normal phase liquid chromatography (LC) was implemented for the purification of a synthetic lipid-peptide conjugate and its electrophilic lipid precursor. N-bromoacetic distearoylphosphatidylethanolamine (DSPE-COCH-2Br) was extracted from the crude reaction mixture and purified in a single chromatographic step with a gradient of chloroform, **methanol**, and 50% aqueous **acetic acid.** This compound was covalently linked to Ser-Phe-Leu-Leu-Arg-Asn(beta-Ala)-3-Tyr-NHCH-2CH-2SH, a hydrophilic **decapeptide**, and the conjugate was both extracted and purified in a single step by normal phase gradient LC using a hand-packed 3 mL Sep-Pak column. The eluent was a mixture of **hexane**, chloroform, 2-**propanol**, **acetic acid**, and **water.** Runs were monitored by thin layer chromatography and the plates developed with iodine and ninhydrin.

AB Conventional normal phase liquid chromatography (LC) was implemented for the purification of a synthetic lipid-peptide conjugate and its electrophilic lipid precursor. N-bromoacetic distearoylphosphatidylethanolamine (DSPE-COCH-2Br) was extracted from the crude reaction mixture and purified in a single chromatographic step with a gradient of chloroform, **methanol**, and 50% aqueous **acetic acid.** This compound was covalently linked to Ser-Phe-Leu-Leu-Arg-Asn(beta-Ala)-3-Tyr-NHCH-2CH-2SH, a hydrophilic **decapeptide**, and the conjugate was both extracted and purified in a single step by normal phase gradient LC using a hand-packed 3 mL Sep-Pak column. The eluent was a mixture of **hexane**, chloroform, 2-**propanol**, **acetic acid**, and **water.** Runs were monitored by thin layer chromatography and the plates developed with iodine and ninhydrin.

L39 ANSWER 5 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1984:207537 BIOSIS
DOCUMENT NUMBER: PREV198477040521; BA77:40521
TITLE: REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC SEPARATION OF THE ALPHA ISOMERS AND BETA ISOMERS OF ASPARTYL PEPTIDES.
AUTHOR(S): JIN H-L [Reprint author]; LIN Y; LI C-X; YIEH Y-H; CHI A-H; LU Y-J; ZHAO C; JIN H-Z; HSING C-Y
CORPORATE SOURCE: DEP CHEMISTRY, BEIJING UNIV BEIJING, PEOPLE'S REPUBLIC CHINA
SOURCE: Chromatographia, (1983) Vol. 17, No. 4, pp. 205-208. CODEN: CHRGB7. ISSN: 0009-5893.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
AB A high-performance liquid chromatography method is described for the separation of the α- and β-isomers of aspartyl-containing peptides such as N-benzyloxycarbonyl methyl aspartate, protected sweet dipeptide and delta sleep inducing peptide (DSIP, a **nonapeptide**). The α- and β-isomers were separated on a column containing octadecyl silica bonded-phase packing under reversed-phase conditions using **methanol-water** as the mobile phase containing a small amount of **acetic acid.** The resolution achieved meets the requirements of qualitative and quantitative analysis. This method is also suited for the determination of intermediates in the aspartyl peptide synthesis.
AB A high-performance liquid chromatography method is described for the separation of the α- and β-isomers of aspartyl-containing peptides such as N-benzyloxycarbonyl methyl aspartate, protected sweet dipeptide and delta sleep inducing peptide (DSIP, a **nonapeptide**). The α- and β-isomers were separated on a column containing octadecyl silica bonded-phase packing under reversed-phase conditions using **methanol-water** as the mobile phase containing a small amount of **acetic acid.** The resolution achieved meets the requirements of qualitative and quantitative analysis. This method is also suited for the determination of intermediates in the aspartyl peptide synthesis.

L39 ANSWER 6 OF 8 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1982:206062 BIOSIS
DOCUMENT NUMBER: PREV198273066046; BA73:66046
TITLE: SYNTHESIS AND CARBON-13 NMR SPECTRA OF THE AMINO TERMINAL DECA PEPTIDE OF THE SEQUENCE OF HUMAN LYMPHO BLAST INTERFERON.
AUTHOR(S): JUNG G [Reprint author]; BRUECKNER H
CORPORATE SOURCE: INSTITUT FUER ORGANISCHE CHEMIE DER UNIV, TUEBINGEN, AUF DER MORGENSTELLE 18, D-7400 TUEBINGEN
SOURCE: Hoppe-Seyler's Zeitschrift fuer Physiologische Chemie, (1981) Vol. 362, No. 3, pp. 292-304. CODEN: HSZPAZ. ISSN: 0018-4888.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: GERMAN
AB The N-terminal sequence 1-10 of intereron HuIFN-α(Ly) from human lymphoblasts, Ser-Asp-Leu-Pro-Gln-Thr-His-Ser-Leu-Gly (LIF[1-10]), was synthesized by the Merrifield method. N-tert-butyloxycarbonylglycin was esterified via its Cs salt with a chloro-methylated polystyrene-1% divinylbenzene support yielding a loading of 0.3 mmol/g. Double couplings, each with a 5-fold excess of N-protected amino acid, were

performed with N,N'-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole, followed by an acetylation step. N-tert-butyloxycarbonyl-L-amino acids with O-benzyl protection for serine, threonine and Nim, and 2,4-dinitrophenyl protection for histidine, and N-fluorenylmethyloxycarbonylaspartic acid β-tert-butyl ester were used. N-tert-butyloxycarbonyl-glutamine was coupled as 4-nitrophenyl ester in the presence of 1-hydroxybenzotriazole. The butyloxycarbonyl groups of the residues 3-10 were removed with trifluoroacetic acid in dichloromethane; the 9-fluorenylmethyloxycarbonyl group was split off with diethylamine. After quantitative hydrazinolysis in dimethylformamide, chromatography on Sephadex LH-20 with **methanol** and reversed-phase chromatography on silica gel RP-8 with **methanol** /H2O 9:1, the **decapeptide** hydrazide Boc-Ser(Bzl)-Asp(But)-Leu-Pro-Gln-Thr(Bzl)-His-Ser(Bzl)-Leu-Gly-NH-NH2 was isolated in pure state. The partially protected **decapeptide** was characterized by 13C-NMR spectroscopy, analyzed and linked with poly(L-lysine) (MW 37,300) via its azide and also using m-xylylene diisocyanate. After a deprotection step the polylysine-LIF[1-10] antigens were dialyzed and lyophilized. The free **decapeptide** LIF[1-10] was split-off from the resin using HBr/CF3CO2H, followed by mercaptoethanol treatment. After purification on Sephadex G-15 with 0.1 M **acetic acid** and on the reversed-phase silica gel RP-8 with **methanol**/H2O 9:1, **water**-soluble LIF[1-10] was obtained in pure state as shown by TLC, electrophoresis, amino acid analysis and 13C-NMR spectroscopy.

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L39 ANSWER 7 OF 8 MEDLINE on STN
ACCESSION NUMBER: 81190268 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6164624
TITLE: [Synthesis and 13C-NMR spectra of the N-terminal **decapeptide** sequence of human lymphoblast

interferon].
Synthese und 13C-NMR-Spektren des N-terminalen Decapeptids der Sequenz von menschlichem Lymphoblasten-Interferon.

AUTHOR: Jung G; Bruckner H
SOURCE: Hoppe-Seyler's Zeitschrift fur physiologische Chemie, (1981 Mar) 362 (3) 291-304.
Journal code: 2985060R. ISSN: 0018-4888.
PUB. COUNTRY: GERMANY, WEST: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: German
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198107
ENTRY DATE: Entered STN: 19900316
Last Updated on STN: 19900316
Entered Medline: 19810709

AB The N-terminal sequence 1-10 of interferon HuIFN-alpha(Ly) from human lymphoblasts Ser-Asp-Leu-Pro-Gln-Thr-His-Ser-Leu-Gly (LIF[1-10]) was synthesized by the Merrifield method. N-tert-Butyloxycarbonylglycin was esterified via its cesium salt with a chloro-methylated polystyrene-1% divinylbenzene support yielding a loading of 0.3 mmol/g. Double couplings, each with a five-fold excess of N-protected amino acid, were performed with N,N'-dicyclohexylcarbodiimide and 1-hydroxybenzotriazole, followed by an acetylation step. N-tert-Butyloxycarbonyl-L-amino acids with O-benzyl protection for serine, threonine, and Nim-2,4-dinitrophenyl protection for histidine, and N-fluorenylmethyloxycarbonylaspartic acid beta-tert-butyl ester were used. N-tert-Butyloxycarbonyl-glutamine was coupled as 4-nitrophenyl ester in the presence of 1-hydroxybenzotriazole. The butyloxycarbonyl groups of the residues 3 to 10 were removed with trifluoroacetic acid in dichloromethane; the 9-fluorenylmethyloxycarbonyl group was split off with diethylamine. After quantitative hydrazinolysis in dimethylformamide, chromatography on Sephadex LH-20 with **methanol** and reversed-phase chromatography on silica gel RP-8 with **methanol/water** 9:1, the **decapeptide** hydrazide Boc-Ser(Bzl)-Asp(But)-Leu-Pro-Gln-Thr(Bzl)-His-Ser(Bzl)-Leu-Gly-NH-HN2 was isolated in pure state. The partially protected **decapeptide** was characterized by 13C-NMR spectroscopy, analysed, and linked with poly(L-lysine) (molecular mass 37 300) via its azide and also using m-xylylene diisocyanate. After a deprotection step the polylysine-LIF[1-10] antigens were dialyzed and lyophilized. Furthermore the free **decapeptide** LIF[1-10] was split-off from the resin using HBr/CF3CO2H, followed by mercaptoethanol treatment. After purification on Sephadex G-15 with 0.1 M **acetic acid** and on the reversed-phase silicagel RP-8 with **methanol/ water** 9:1 **water** soluble LIF-[1-10] was obtained in pure state as shown by thin-layer-chromatography, electrophoreses amino acid analysis and 13C-NMR spectroscopy.

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L39 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

ACCESSION NUMBER: 1963:469442 CAPLUS
DOCUMENT NUMBER: 59:69442
ORIGINAL REFERENCE NO.: 59:12923b-f,12924a
TITLE: Cyclic octapeptides
PATENT ASSIGNEE(S): Farbwerke Hoechst A.-G.
SOURCE: 6 pp.
DOCUMENT TYPE: Patent
LANGUAGE: Unavailable
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
GB 927714		19630606	GB	
DE 1193509			DE	
PRIORITY APPLN. INFO.:			DE	19600602

AB The new octapeptides exhibit a valuable vasopressor action comparable to that exhibited by lysine-vasopressin but without the undesired ocytocic side effects. A tripeptide azide is treated with a hexapeptide to form a **nonapeptide.** This latter compound after splitting off the protective groups, is converted by oxidation with air at room temperature in aqueous solution at pH 6.5 to 8.5 to the desired octapeptide. N-Carbobenzoxy-S-benzyl-L-cysteinyl-O-methyl-L-tyrosyl-L-phenylalanine azide (I) (7.5 g.) and L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-Nε-tosyl-L-lysylglycinamide (II) (8.1 g.) are dissolved at room temperature in 120 ml. HCONMe2. The material is maintained at 0° overnight and then two days at 20°, concentrated to 60 ml., and shaken with 500 ml. EtOAc. The moist **nonapeptide** is then triturated with 200 ml. **methanol**, filtered, and washed with **methanol**, EtOAc, and ether. After drying, 7.6 g. N-carbobenzoxy-S-benzyl-L-cysteinyl-O-methyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-as-paraginyl-S-benzyl-L-cysteinyl-L-prolyl-Nε-tosyl-L-lysylglycinamide (III) is obtained as a powder, m. 209-11°, [α]20D 31.1° (c 2, HCONMe2). To 3.08 g. I in 2000 ml. NH3 with Na chips is added 1.0 g. NH4Cl and the NH3 distilled The residue is taken up in 2000 ml. 0.2% **acetic acid** and the solution adjusted to pH 8.0 with 5% AcOH. After standing for 3 hrs. under CO2-free air, the solution is filtered. The

colorless filtrate is adjusted to pH 4.5 with glacial **acetic acid** and lyophilized. The crude O-methyl-tyrosine2-lysine8-vasopressin (IV) is purified by countercurrent distribution between sec-BuOH and 0.08M p-MeC6H4SO3H. The biologically active fraction with a distribution coeficient K = 0.82 has an activity of 25 ± 15 I.U. of vasopressin activity and 0.2 ± 0.5 I.U. of oxytocin activity. The Rf value is 0.33 (65:15:20 MeCOEt-C5H5N-H2O). I is obtained by preparing N-carbobenzoxy-S-benzyl-L-cysteinyl-O-methyl-L-tyrosyl-L-phenylalanine Me ester (V), m. 167-8°, from N-carbobenzoxy- S-benzyl-L-cysteinyl-O-methyl-L-tyrosine and L-phenylalanine Me ester-HCl with Et3N and dicyclohexylcarbodiimide in chloroform. V in **ethanol** with N2H4.H2O on standing overnight yields N-carbobenzoxy-S-benzyl-L-cysteinyl-O-methyl-L-tyrosyl-L-phenylalanine hydrazide (VI), m. 219-20°. I is prepared by treating VI with NaNO2 solution in AcOH and HCl with cooling and the product precipitated in ice **water.** Similarly, 8.3 g. V in 100 ml. dioxane is stirred at room temperature with 15 ml. NaOH and 20 ml. **methanol.** After 2 hrs., 15.2 ml. H2SO4 is added and the solvent removed in vacuo. The residue is taken up in EtOAc, washed with **water**, dried, and evaporated The free acid residue of 7.7 g. V is obtained. This residue is taken up in 60 ml. HCONMe2 and 8.9 g. L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-propyl-Nε-tosyl-L-lysylglycinamide (VII) and 2.1 g. dicyclohexylcarbodiimide added and the mixture is allowed to stand at room temperature for two days. It is heated to 40° to give 7.5 g. III, [α]20D -38°(c 2 HCONMe2).

Also, L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzylcysteinyl-L-propyl-Nε-tosyl-L-lysylglycinamide and N-Carbobenzoxy-O-methyl-L-tyrosine nitrophenyl ester in HCONMe2-EtOAc yields a semisolid, which is washed with **ethanol** and EtOAc and then suspended in glacial AcOH and 4N HBr. After 2 hrs., dry ether is added, the separated hydrobromide dissolved in HCONMe2, and Et3N and N-carbobenzoxy-S-benzyl-L-cysteine nitrophenyl ester added. After two days, EtOAc is added and III filtered off, m. 210-13°, [α]20D -39.5° (c 2, HCONMe2). The reaction of 7.5 g. N-carbobenzoxy-S-benzyl-L-cysteinyl-O-ethyl-L-tyrosyl-L-phenylalanine azide and 8.1 g. VII gives N-carbobenzoxy-S-benzyl-L-cysteinyl-O-ethyl-L-tyrosyl-L-Nε-toxyl-L-lysylglycinamide, m. 216-18°, [α]20D 39.1°. When treated with ammonia and sodium, followed by oxidation, this is converted into O-ethyl-tyrosine2-lysine8-vasopressin, distribution coefficient K 0.84, Rf 0.35.

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=> dis his

(FILE 'HOME' ENTERED AT 15:35:23 ON 09 DEC 2005)

FILE 'REGISTRY' ENTERED AT 15:36:15 ON 09 DEC 2005
E NONAPEPTIDE/CN 5
E DECAPEPTIDE/CN 5
L1 5 S (METHANOL OR ETHANOL OR PROPANOL OR ISOPROPANOL)/CN
L2 14 S (METHYL ACETATE OR ETHYL ACETATE OR METHYL PROPIONATE OR ETHY
L3 2 S (".TERTIARY.- BUTYL ACETATE" OR ACETIC ACID OR PROPIONIC ACID

FILE 'MEDLINE, BIOSIS, EMBASE, CAPLUS' ENTERED AT 15:39:41 ON 09 DEC 2005
L4 23205 FILE MEDLINE
L5 34113 FILE BIOSIS
L6 39078 FILE EMBASE
L7 417772 FILE CAPLUS
TOTAL FOR ALL FILES
L8 514168 S L2 OR L3 OR METHYL ACETATE OR ETHYL ACETATE OR METHYL PROPION
L9 33366 FILE MEDLINE
L10 46001 FILE BIOSIS

Prepared by: Mary Hale @2-2507 Rem Bldg 1D86

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L11        44680 FILE EMBASE
L12       223501 FILE CAPLUS
     TOTAL FOR ALL FILES
L13       347548 S ACETIC ACID OR PROPIONIC ACID
L14        50602 FILE MEDLINE
L15        69093 FILE BIOSIS
L16        64225 FILE EMBASE
L17       544494 FILE CAPLUS
     TOTAL FOR ALL FILES
L18       728414 S L8 OR L13
L19       103185 FILE MEDLINE
L20       172173 FILE BIOSIS
L21       141509 FILE EMBASE
L22       506717 FILE CAPLUS
     TOTAL FOR ALL FILES
L23       923584 S L1 OR METHANOL OR ETHANOL OR PROPANOL OR ISOPROPANOL

     FILE 'CAPLUS' ENTERED AT 15:41:51 ON 09 DEC 2005
               E NONAPEPTIDE/CT
               E E3+ALL
               E DECAPEPTIDE/CT
               E E4+ALL

     FILE 'MEDLINE, BIOSIS, EMBASE, CAPLUS' ENTERED AT 15:42:30 ON 09 DEC 2005
L24         3301 FILE MEDLINE
L25         3454 FILE BIOSIS
L26         3129 FILE EMBASE
L27         5580 FILE CAPLUS
     TOTAL FOR ALL FILES
L28        15464 S NONAPEPTIDE? OR DECAPEPTIDE?
L29           11 FILE MEDLINE
L30           17 FILE BIOSIS
L31            9 FILE EMBASE
L32           17 FILE CAPLUS
     TOTAL FOR ALL FILES
L33           54 S L28 AND (DISSOL? SOLVENT? OR (WATER AND L23))
L34            3 FILE MEDLINE
L35            5 FILE BIOSIS
L36            3 FILE EMBASE
L37            5 FILE CAPLUS
     TOTAL FOR ALL FILES
L38           16 S L33 AND L18
L39            8 DUP REM L38 (8 DUPLICATES REMOVED)

=> s purif? and l28 and (precipit? solvent or l18)
L40           15 FILE MEDLINE
L41           12 FILE BIOSIS
L42            8 FILE EMBASE
L43           22 FILE CAPLUS

TOTAL FOR ALL FILES
L44           57 PURIF? AND L28 AND (PRECIPIT? SOLVENT OR L18)

=> s l44 not l38
L45           13 FILE MEDLINE
L46            9 FILE BIOSIS
L47            6 FILE EMBASE
L48           18 FILE CAPLUS

TOTAL FOR ALL FILES
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L49 46 L44 NOT L38

=> s l49 and isolat? and dry?
L50 0 FILE MEDLINE
L51 0 FILE BIOSIS
L52 0 FILE EMBASE
L53 3 FILE CAPLUS

TOTAL FOR ALL FILES
L54 3 L49 AND ISOLAT? AND DRY?

=> d 1-3 ibib abs hit;s l49 not l54

L54 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1965:22810 CAPLUS
DOCUMENT NUMBER: 62:22810
ORIGINAL REFERENCE NO.: 62:4118b-h,4119a-h,4120a-d
TITLE: Peptides synthesis. XXV. The synthesis of Pro1-Arg2-, Ala4-,Ser4-Gly6-,Tyr5-, and Orn9-bradykinin
AUTHOR(S): Schroeder, Eberhard; Petras, Hans Siegfried; Klieger, Erich
CORPORATE SOURCE: Schering A.-G., Berlin
SOURCE: Ann. (1964), 679, 221-31
DOCUMENT TYPE: Journal
LANGUAGE: German
AB The synthesis of the 5 title bradykinin analogs is described. Their biol. properties are compared with those of bradykinin and other known bradykinin analogs. Z-Pro-(O2N)Arg (0.645 g.) in 5 cc. DMF combined with Pro-Gly-Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p (from 1.3 millimoles HBr salt and 0.2 cc. Et3N) in 10 cc. DMF, 0.295 g. DCC in 2 cc. THF added at 0°, the mixture kept 48 hrs. at room temperature filtered, and concentrated, the residue dissolved in EtOAc-BuOH, and the solution washed 5 times with N HCl, 5 times with saturated aqueous NaHCO3, and H2O and concentrated in vacuo gave 1.2 g. Z-Pro-(O2N)Arg-Pro-Gly-Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4 NO2-p, amorphous, m. 160-5° (Me2CO-iso-Pr2O), [α]25D-40.9° (c 0.5, DMF), which (1.0 g.) hydrogenated in 8:1:1 MeOH-AcOH-H2O over Pd black, the filtered solution concentrated in vacuo, the residue dissolved in H2O and lyophilized, the product **purified** by chromatography on a CMC column with gradient elution (0.001 to 0.125M) with NH4OAc buffer (pH 5.5), and the eluate lyophilized gave 375 mg. Pro-Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg.1.5AcOH.5H2O (Pro1-Arg2-bradykinin) (I), [α]23D -67.4° (c 0,5, H2O). Z-Pro-Ala-OMe (7.01 g.) in 50 cc. MeOH stirred 3 hrs. at room temperature with 23.1 cc. N NaOH, MeOH removed in vacuo, the residual solution diluted with 25 cc. H2O, extracted 3 times with EtOAc, acidified with HCl, and extracted with EtOAc, and the extract washed neutral, dried, and evaporated gave 5.3 g. Z-Pro-Ala(II), m. 161-2°(H2O), [α]20D-57.9° (c 2, MeOH). II(5.28 g.) in 75 cc. THF combined with 2.25g. BOC-NHNH2 (III) in 25 cc. THF, the solution cooled to -15°, treated with 3.71 g. DCC in 5 cc. THF with stirring, kept 24 hrs. at 0°, treated with 1 cc. 5N AcOH, kept 60 min. at room temperature, filtered, and concentrated, the residue dissolved in EtOAc, and the solution washed with 10% aqueous citric acid, H2O, aqueous NaHCO3, and H2O, dried, and concentrated gave 3.5 g. Z-Pro-Ala-NHNH-BOC (IV), m. 93-5°(Et2O at -10°), [α]22D -93.4° (c 2, MeOH). II(5.44 g.) in 75 cc. THF treated with 2.35 cc. Et3N and 1.73cc. ClCO2Et at -10°, after 10 min. 2.25 g. III added, and the mixture warmed slowly to room temperature with stirring,

kept 18 hrs. at room temperature, and worked up gave 3.5 g. IV, m. 93.5°, [α]24D-93.9°(c 2, MeOH). IV (3.38g.) hydrogenated in MeOH over Pd black and the filtered solution concentrated gave 2.19 g. Pro-Ala-NHNH-BOC (V), m. 96-8° (EtOAc-iso-Pr2O), [α]24D -86.5° (c 1, MeOH). Z-(O2N)Arg-Pro (Va) (6.75 g.) in 30 cc. DMF combined with 4.5 g. V in 30 cc. MeCN, the solution diluted with 20 cc. THF, cooled to -10°, treated dropwise with 3.31 g. DCC in 10 cc. THF with stirring, kept 24 hrs. at 0°, treated with 5N AcOH, kept 60 min. at room temperature, filtered, and concentrated, the residue dissolved in CHCl3, and the solution washed with 10% aqueous citric acid, H2O, saturated aqueous NaHCO3, and H2O, dried, concentrated almost to **dryness**, and diluted iso-Pr2O gave 5.0 g. Z-(O2N)Arg-Pro-Pro-Ala-NHNH-BOC, m. 148-50° (CHCl3-iso-Pr2O), [α]22D -112.5° (c 1, MeOH), which (2.49 g.) kept 1 hr. at room temperature in 100 cc. 20% absolute EtOH-HCl and the solution concentrated to **dryness** gave 2.3 g. Z-(O2N)Arg-Pro-Pro-Ala-NHNH2.HCl (VI.HCl), m. 140-2° (decomposition) (MeOH-Et2O), [α25D -105.8° (c 1, MeOH). Z-Pro-Ala-NHNH2 (2.34 g.) in 5 cc. DMF and 7 cc. 2N THF-HCl treated with 0.87 cc. tert-BuONO at -15°, after 5 min. the solution adjusted to pH 7.0 with Et3N, treated with 5.82 g. Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p (VIa) in 20 cc. DMF, kept 2 days at 0° and 1 day at room temperature, and evaporated in vacuo, the residue dissolved in 10:1:1 EtOAc-BuOH-CHCl3, and the solution washed 4 times with N HCl, saturated aqueous NaHCO3 and saturated aqueous NaCl, dried, concentrated in vacuo, and diluted with Bu2O gave after 2 repptns. from CHCl3-**cyclohexane** 5.6 g. Z-Pro-Ala-Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p, [α]23D -42.9° (c 1, DMF), which (4.55 g.) treated in 10 cc. EtOAc (suspension) with 10 cc. 37% EtOAc-HBr and after 3.5 hrs. 200 cc. Et2O added gave 4.35 g. Pro-Ala-Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p.2.5HBr (VII.2.5HBr). VII.2.5HBr (4.21 g.) in 20 cc. DMF treated at -15° with 1.21 cc. Et3N and then with 1.57 g. Va in 20 cc. DMF, 792 mg. DCC in 5 cc. THF added with stirring, the solution kept 22 hrs. at 0°, treated with 5N AcOH, kept 2 hrs. at room temperature, filtered, and evaporated in vacuo, the residue dissolved in 10:2:2 EtOAc-BuOH-CHCl3, and the solution washed 4 times with N HCl, saturated aqueous NACl, saturated aqueous NaHCO3, and saturated aqueous NaCl, dried, and diluted with Bu2O, the precipitate filtered off and dissolved in 10:1:1 MeOH-EtOH-H2O, the solution stirred 6 hrs. with 5 cc. Dowex-50 (H+ form),the resin filtered off and washed with MeOH, and the combined filtrate and washings evaporated in vacuo gave after repptn. from CHCl3-**cyclohexane** 2.6 g. Z-(O2N)Arg-Pro-Pro-Ala-Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p (VIII), [α]22D -55.1° (c 1, DMF). VI.HCl (1.37 g.) in 2 cc. 2N THF-HCl and 2 cc. DMF stirred 10 min. at -10° with 0.25 cc. tert-BuONO, the solution adjusted to pH 7.0 with Et3N, treated with 1.65 g. VIa in 10 cc. DMF, kept 48 hrs. at 0° and 24 hrs. at room temperature, and concentrated to **dryness**, the residue dissolved in 3:1 CHCl3-BuOH, and the solution washed with N HCl, saturated aqueous NaHCO3, and H2O, dried, concentrated, and diluted with **cyclohexane** gave 2.1 g. VIII, [α]25D -55.6° (c 1, DMF). VIII (1.86 g.) hydrogenated in 6:1:1 MeOH-AcOH-H2O over Pd black, the solution filtered and concentrated, the residue dried over KOH, dissolved in H2O, and lyophilized, and the product **purified** by chromatography and electrophoresis gave after

lyophilizing 380 mg. Arg-Pro-Pro-Ala-Phe-Ser-Pro-Phe-Arg.1.5AcOH.10H2O (Ala4-bradykinin) (IX), [α]22D -99.0° (c 1, H2O). Z-Tyr-Ser-OMe (10.4 g.) in 20 cc. DMF and 20 cc. EtOH kept 24 hrs. at room temperature with 5 cc. N2H4.H2O gave 10.2 g. hydrazide, m. 226-8° (DMF-EtOH), [α]22D -5.3° (c 1, DMF), which (1.66 g.) in 2 cc. DMF and 3.8 cc. 2.2N THF-HCl treated with 0.49 cc. tert-BuONO at -15°, after 5 min. the solution diluted with 5 cc. DMF, neutralized with Et3N, filtered, combined with Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p (from 4 millimoles HBr salt and Et3N) in 6 cc. DMF, kept 24 hrs. at 0° and 24 hrs. at room temperature, and concentrated, the residue dissolved in BuOH-EtOAc, and the solution washed as usual and concentrated gave after repptn. from EtOH-Et2O 2.95 g. Z-Tyr-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p (X), [α]22D -32.8° (c 0.5, DMF). X (1.97 g.) in 60 cc. CF3CO2H treated with HBr 40 min. at 0° and concentrated in vacuo and the residue triturated with Et2O gave 1.9 g. Tyr-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p.1.5HBr (XI.HBr), [α]22D -35.6° (c 0.5, MeOH). To 1.31 g. Z-(O2N)Arg-Pro-Pro-Gly-NHNH2.HCl(XIa.HCl) in 3 cc. DMF and 1.9 cc. 2.2N THF-HCl was added dropwise 0.244 cc. tert-BuONO at -15° and after 5 min. the solution diluted with DMF, neutralized with Et3N, filtered, combined with XI (from 2 millimoles XI.HBr and Et3N) in 6 cc. DMF, kept 24 hrs. at 0° and 24 hrs. at room temperature, and worked up to give after repptn. from EtOH-Et2O 1.9 g. amorphous Z-(O2N)Arg-Pro-Pro-Gly-Tyr-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p, [α]22D -46.4° (c 1, DMF), which (1.43 g.) hydrogenated in MeOH-AcOH-H2O over Pd black, the filtered solution concentrated, and the residue dissolved in H2O and lyophilized gave 1.2 g. crude product, which (0.75 g.) **purified** by electrophoresis gave 0.297 g. Arg-Pro-Pro-Gly-Tyr-Ser-Pro-Phe-Arg.2AcOH.4.5H2O (Tyr5-bradykinin) (XII), [α]22D -76.0° (c 0.5, H2O). Z-Pro-Ser-NHNH2 (cf. initial part)(0.7 g.)in 4.8 cc. AcOH, 3 cc. 2N HCl, and 5 cc. H2O treated with 0.14 g. NaNO2 in H2O at 0°, the azide extracted with EtOAc, and the extract washed neutral with saturated aqueous NaHCO3, dried, combined with Phe-Gly-Pro-Phe-(O2N)Arg-OMe (from 2 millimoles HBr salt and Et3N) in 5 cc. DMF, kept 24 hrs. at 0° and 24 hrs. at room temperature, and worked up gave 1.3 g. Z-Pro-Ser-Phe-Gly-Pro-Phe-(O2N)Arg-OMe, [α]22D -67.6° (c 0.5, DMF), which (1.0 g.) in 15 cc. CF3CO2H treated with HBr 90 min. at 0°, the solution evaporated in vacuo, and the residue triturated with Et2O gave 0.95 g. amorphous Pro-Ser-Phe-Gly-Pro-Phe-(O2N)Arg-OMe.1.5HBr.0.5H2O (XIII.HBr), [α]22D -43.6° (c 0.5, DMF). Z-(O2N)Arg-Pro.THF (0.523 g.) in 3 cc. DMF combined at -10° with XIII (from 1 millimole XIII.HBr and Et3N) in 4 cc. DMF and with 0.309 g. DCC in THF and the mixture kept 2 hrs. at 0° and 20 hrs. at room temperature and worked up gave 0.6 g. Z-(O2N)Arg-Pro-Pro-Ser-Phe-Gly-Pro-Phe-(O2N)-Arg-OMe (XIV), [α]22D -59.4° (c 0.5, DMF). XIV (0.455 g.) in 3 cc. dioxane and 1 cc. EtOH stirred 2.5 hrs. with 0.7 cc. N NaOH, the solution evaporated, and the residue dissolved in H2O and acidified with N HCl gave 0.330 g. corresponding benzyloxycarbonylnonapeptide acid, [α]22D -61.8° (c 0.5, DMF), which (270 mg.) hydrogenated in MeOH-AcOH-H2O over Pd black, the filtered solution evaporated in vacuo, and the residue dissolved in H2O and lyophilized gave 250 mg. product, which **purified** by preparative electrophoresis in C5H5N.AcOH buffer (pH 5) gave 131 mg. Arg-Pro-Pro-Ser-Phe-Gly-Pro-Phe-Arg.1.5AcOH.4H2O (Ser4-Gly6-bradykinin) (XV), [α]22D -96.0° (c 0.5, H2O). From δ-benzylideneornithine and PhCH2O2CCl was prepared 71% Nα-(benzyloxycarbonyl)ornithine (XVI), m. 209-10° (H2O), [α]25D -8.4° (c 1.5, N HCl). XVI (74.5 g.) in 370 cc. H2O and 240 cc. dioxane shaken 4 days at room temperature with 43 g. III and 17 g. MgO in a pressure flask, the solution filtered, concentrated in vacuo to .apprx.1/4

its volume, diluted with H2O, and acidified to pH 2-3 with 20% citric acid, and the oily product (101 g.) **isolated** with EtOAc and recrystd. from EtOAc-petr. ether gave 70 g. Nα-(benzyloxycarbonyl)-Nδ-(tert-butoxycarbonyl)ornithine (XVII), m. 97-8°, [α]25D -8.8° (c 1, C5H5N); dicyclohexylammonium salt m. 128-9° (EtOAc), [α]25D 7.8° (c 1, EtOH). XVII (68 g.) in Et2O-MeOH added dropwise to 900 cc. Et2O-CH2N2 (from 72 g. H2NCONMeNO) at 0° with stirring and the solution kept overnight and worked up gave 63 g. XVII Me ester, m. 70-1° (Et2O-petr. ether), [α]25D -13.3° (c 1, MeOH), which (3.0 g.) hydrogenated in 80 cc. MeOH over Pd black, the filtered solution concentrated, and the residual oily ester treated in EtOAc-Et2O with Et2O-HCl at -20° gave 1.5 g. Nδ-(tert-butoxycarbonyl)ornithine Me ester-HCl (XVIII.HCl), m. 154-5° (MeOH-Et2O-petr. ether), [α]25D 15.5° (c 1, MeOH). Z-Pro-Phe-NHNH2 (15.0 g.) in 35 cc. 2.2N THF-HCl treated with 4.5 cc. tert-BuONO at -25° with stirring and the solution stirred 10 min. at -25°, diluted with 400 cc. cold (-25°) EtOAc, washed with ice cold saturated aqueous NaHCO3 and H2O, dried, treated with a cold solution of XVIII (from 11.3 g. XVIII.HCl and 5.7 cc. Et3N in CHCl3) at .apprx.-20°, kept 24 hrs. at 0° and 24 hrs. at room temperature, and worked up gave 17.7 g. Z-Pro-Phe-(Nδ-BOC)-Orn-OMe(XIX), m. 120-2° (EtOAc-petr. ether), [α]25D -52.8° (c 1, MeOH), which (12.5 g.) hydrogenated in 200 cc. MeOH over Pd black and the filtered solution concentrated gave 9.1 g. Pro-Phe-(Nδ-BOC)-Orn-OMe (XX), foam, [α]25D -25.6° (c 1, MeOH). Z-Phe-Ser-NHNH2 (6.8 g.) in 20 cc. DMF and 16.1 cc. 2.2N THF-HCl treated like XIX with 2.1 cc. tert-BuONO and the EtOAc solution of the azide treated like XIX with XX (in this case concentration of the EtOAc phase gave crystalline product) yielded 9.4 g. Z-Phe-Ser-Pro-Phe-(Nδ-BOC)-Orn-OMe, m. 162-4° (EtOAc-EtOH-petr. ether, EtOH), [α]25D -43.1° (c 1, AcOH), which (8.6 g.) hydrogenated in 100 cc. MeOH over Pd black gave 4.2 g. Phe-Ser-Pro-Phe-(Nδ-BOC)-Orn-OMe (XXI), m. 152-3° (EtOAc-petr. ether), [α]25D -58.3° (c 0.5, MeOH). XIa.HCl (1.64 g.) in 2 cc. DMF treated at -20° with 2.4 cc. 2.2N THF-HCl and 0.31 cc. tert-BuONO and after 5-10 min. the solution diluted with 5 cc. DMF, neutralized with Et3N, filtered, combined with XXI in 3 cc. DMF, kept 24 hrs. at 0° and 24 hrs. at room temperature, and worked up gave 1.6 g. Z-(O2N)-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-(Nδ-BOC)-Orn-OMe (XXII), [α]22D -52.4° (c 0.5, DMF). XXII (1.31 g.) in 10 cc. dioxane stirred 2.5 hrs. with 2 cc. N NaOH, concentrated, diluted with H2O, and acidified with 10% aqueous citric acid at 0° gave 1.0 g. corresponding protected **nonapeptide** acid, [α]23D -51.4° (c 0.5, DMF), which (984 mg.) hydrogenated in MeOH-AcOH-H2O over Pd black, the filtered solution concentrated in vacuo, the residue treated 75 min. with 3 cc. CF3CO2H, and the solution diluted with cold Et2O gave 0.95 g. product, which (540 mg.) **purified** by preparative electrophoresis gave 314 mg. Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Orn.2AcOH.5H2O (Orn9-bradykinin) (XXIII), [α]22D -86.0° (c 0.5, H2O). The biol. properties of the bradykinin analogs (discussed) were considerably below the biol. activity of bradykinin.

AB The synthesis of the 5 title bradykinin analogs is described. Their biol. properties are compared with those of bradykinin and other known bradykinin analogs. Z-Pro-(O2N)Arg (0.645 g.) in 5 cc. DMF combined with Pro-Gly-Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p (from 1.3 millimoles HBr salt and 0.2 cc. Et3N) in 10 cc. DMF, 0.295 g. DCC in 2 cc. THF added at 0°, the mixture kept 48 hrs. at room temperature filtered, and concentrated, the

residue dissolved in EtOAc-BuOH, and the solution washed 5 times with N HCl, 5 times with saturated aqueous NaHCO3, and H2O and concentrated in vacuo gave 1.2 g.

Z-Pro-(O2N)Arg-Pro-Gly-Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4 NO2-p, amorphous, m. 160-5° (Me2CO-iso-Pr2O), [α]25D-40.9° (c 0.5, DMF), which (1.0 g.) hydrogenated in 8:1:1 MeOH-AcOH-H2O over Pd black, the filtered solution concentrated in vacuo, the residue dissolved in H2O and lyophilized, the product **purified** by chromatography on a CMC column with gradient elution (0.001 to 0.125M) with NH4OAc buffer (pH 5.5), and the eluate lyophilized gave 375 mg. Pro-Arg-Pro-Gly-Phe-Ser-Pro-Phe-Arg.1.5AcOH.5H2O (Pro1-Arg2-bradykinin) (I), [α]23D -67.4° (c 0,5, H2O). Z-Pro-Ala-OMe (7.01 g.) in 50 cc. MeOH stirred 3 hrs. at room temperature with 23.1 cc. N NaOH, MeOH removed in vacuo, the residual solution diluted with 25 cc. H2O, extracted 3 times with EtOAc, acidified with HCl, and extracted with EtOAc, and the extract washed neutral, dried, and evaporated gave 5.3 g. Z-Pro-Ala(II), m. 161-2°(H2O), [α]20D-57.9° (c 2, MeOH). II(5.28 g.) in 75 cc. THF combined with 2.25g. BOC-NHNH2 (III) in 25 cc. THF, the solution cooled to -15°, treated with 3.71 g. DCC in 5 cc. THF with stirring, kept 24 hrs. at 0°, treated with 1 cc. 5N AcOH, kept 60 min. at room temperature, filtered, and concentrated, the residue dissolved in EtOAc, and the solution washed with 10% aqueous citric acid, H2O, aqueous NaHCO3, and H2O, dried, and concentrated gave 3.5 g. Z-Pro-Ala-NHNH-BOC (IV), m. 93-5°(Et2O at -10°), [α]22D -93.4° (c 2, MeOH). II(5.44 g.) in 75 cc. THF treated with 2.35 cc. Et3N and 1.73cc. ClCO2Et at -10°, after 10 min. 2.25 g. III added, and the mixture warmed slowly to room temperature with stirring, kept 18 hrs. at room temperature, and worked up gave 3.5 g. IV, m. 93.5°, [α]24D-93.9°(c 2, MeOH). IV (3.38g.) hydrogenated in MeOH over Pd black and the filtered solution concentrated gave 2.19 g. Pro-Ala-NHNH-BOC (V), m. 96-8° (EtOAc-iso-Pr2O), [α]24D -86.5° (c 1, MeOH). Z-(O2N)Arg-Pro (Va) (6.75 g.) in 30 cc. DMF combined with 4.5 g. V in 30 cc. MeCN, the solution diluted with 20 cc. THF, cooled to -10°, treated dropwise with 3.31 g. DCC in 10 cc. THF with stirring, kept 24 hrs. at 0°, treated with 5N AcOH, kept 60 min. at room temperature, filtered, and concentrated, the residue dissolved in CHCl3, and the solution washed with 10% aqueous citric acid, H2O, saturated aqueous NaHCO3, and H2O, dried, concentrated almost to **dryness**, and diluted iso-Pr2O gave 5.0 g. Z-(O2N)Arg-Pro-Pro-Ala-NHNH-BOC, m. 148-50° (CHCl3-iso-Pr2O), [α]22D -112.5° (c 1, MeOH), which (2.49 g.) kept 1 hr. at room temperature in 100 cc. 20% absolute EtOH-HCl and the solution concentrated to **dryness** gave 2.3 g. Z-(O2N)Arg-Pro-Pro-Ala-NHNH2.HCl (VI.HCl), m. 140-2° (decomposition) (MeOH-Et2O), [α25D -105.8° (c 1, MeOH). Z-Pro-Ala-NHNH2 (2.34 g.) in 5 cc. DMF and 7 cc. 2N THF-HCl treated with 0.87 cc. tert-BuONO at -15°, after 5 min. the solution adjusted to pH 7.0 with Et3N, treated with 5.82 g. Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p (VIa) in 20 cc. DMF, kept 2 days at 0° and 1 day at room temperature, and evaporated in vacuo, the residue dissolved in 10:1:1 EtOAc-BuOH-CHCl3, and the solution washed 4 times with N HCl, saturated aqueous NaHCO3 and saturated aqueous NaCl, dried, concentrated in vacuo, and diluted with Bu2O gave after 2 repptns. from CHCl3-**cyclohexane** 5.6 g. Z-Pro-Ala-Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p, [α]23D -42.9° (c 1, DMF), which (4.55 g.) treated in 10 cc. EtOAc

(suspension) with 10 cc. 37% EtOAc-HBr and after 3.5 hrs. 200 cc. Et2O added gave 4.35 g. Pro-Ala-Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p.2.5HBr (VII.2.5HBr). VII.2.5HBr (4.21 g.) in 20 cc. DMF treated at -15° with 1.21 cc. Et3N and then with 1.57 g. Va in 20 cc. DMF, 792 mg. DCC in 5 cc. THF added with stirring, the solution kept 22 hrs. at 0°, treated with 5N AcOH, kept 2 hrs. at room temperature, filtered, and evaporated in vacuo, the residue dissolved in 10:2:2 EtOAc-BuOH-CHCl3, and the solution washed 4 times with N HCl, saturated aqueous NACl, saturated aqueous NaHCO3, and saturated aqueous NaCl, dried, and diluted with Bu2O, the precipitate filtered off and dissolved in 10:1:1 MeOH-EtOH-H2O, the solution stirred 6 hrs. with 5 cc. Dowex-50 (H+ form),the resin filtered off and washed with MeOH, and the combined filtrate and washings evaporated in vacuo gave after repptn. from CHCl3-**cyclohexane** 2.6 g. Z-(O2N)Arg-Pro-Pro-Ala-Phe-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p (VIII), [α]22D -55.1° (c 1, DMF).

VI.HCl (1.37 g.) in 2 cc. 2N THF-HCl and 2 cc. DMF stirred 10 min. at -10° with 0.25 cc. tert-BuONO, the solution adjusted to pH 7.0 with Et3N, treated with 1.65 g. VIa in 10 cc. DMF, kept 48 hrs. at 0° and 24 hrs. at room temperature, and concentrated to **dryness**, the residue dissolved in 3:1 CHCl3-BuOH, and the solution washed with N HCl, saturated aqueous NaHCO3, and H2O, dried, concentrated, and diluted with **cyclohexane** gave 2.1 g. VIII, [α]25D -55.6° (c 1, DMF). VIII (1.86 g.) hydrogenated in 6:1:1 MeOH-AcOH-H2O over Pd black, the solution filtered and concentrated, the residue dried over KOH, dissolved in H2O, and lyophilized, and the product **purified** by chromatography and electrophoresis gave after lyophilizing 380 mg. Arg-Pro-Pro-Ala-Phe-Ser-Pro-Phe-Arg.1.5AcOH.10H2O (Ala4-bradykinin) (IX), [α]22D -99.0° (c 1, H2O). Z-Tyr-Ser-OMe (10.4 g.) in 20 cc. DMF and 20 cc. EtOH kept 24 hrs. at room temperature with 5 cc. N2H4.H2O gave 10.2 g. hydrazide, m. 226-8° (DMF-EtOH), [α]22D -5.3° (c 1, DMF), which (1.66 g.) in 2 cc. DMF and 3.8 cc. 2.2N THF-HCl treated with 0.49 cc. tert-BuONO at -15°, after 5 min. the solution diluted with 5 cc. DMF, neutralized with Et3N, filtered, combined with Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p (from 4 millimoles HBr salt and Et3N) in 6 cc. DMF, kept 24 hrs. at 0° and 24 hrs. at room temperature, and concentrated, the residue dissolved in BuOH-EtOAc, and the solution washed as usual and concentrated gave after repptn. from EtOH-Et2O 2.95 g. Z-Tyr-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p (X), [α]22D -32.8° (c 0.5, DMF). X (1.97 g.) in 60 cc. CF3CO2H treated with HBr 40 min. at 0° and concentrated in vacuo and the residue triturated with Et2O gave 1.9 g. Tyr-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p.1.5HBr (XI.HBr), [α]22D -35.6° (c 0.5, MeOH). To 1.31 g. Z-(O2N)Arg-Pro-Pro-Gly-NHNH2.HCl(XIa.HCl) in 3 cc. DMF and 1.9 cc. 2.2N THF-HCl was added dropwise 0.244 cc. tert-BuONO at -15° and after 5 min. the solution diluted with DMF, neutralized with Et3N, filtered, combined with XI (from 2 millimoles XI.HBr and Et3N) in 6 cc. DMF, kept 24 hrs. at 0° and 24 hrs. at room temperature, and worked up to give after repptn. from EtOH-Et2O 1.9 g. amorphous Z-(O2N)Arg-Pro-Pro-Gly-Tyr-Ser-Pro-Phe-(O2N)Arg-OCH2C6H4NO2-p, [α]22D -46.4° (c 1, DMF), which (1.43 g.) hydrogenated in MeOH-AcOH-H2O over Pd black, the filtered solution concentrated, and the residue dissolved in H2O and lyophilized gave 1.2 g. crude product, which (0.75 g.) **purified** by electrophoresis gave 0.297 g. Arg-Pro-Pro-Gly-Tyr-Ser-Pro-Phe-Arg.2AcOH.4.5H2O (Tyr5-bradykinin) (XII), [α]22D -76.0° (c 0.5, H2O). Z-Pro-Ser-NHNH2 (cf.

initial part)(0.7 g.)in 4.8 cc. AcOH, 3 cc. 2N HCl, and 5 cc. H2O treated with 0.14 g. NaNO2 in H2O at 0°, the azide extracted with EtOAc, and the extract washed neutral with saturated aqueous NaHCO3, dried, combined with Phe-Gly-Pro-Phe-(O2N)Arg-OMe (from 2 millimoles HBr salt and Et3N) in 5 cc. DMF, kept 24 hrs. at 0° and 24 hrs. at room temperature, and worked up gave 1.3 g. Z-Pro-Ser-Phe-Gly-Pro-Phe-(O2N)Arg-OMe, [α]22D -67.6° (c 0.5, DMF), which (1.0 g.) in 15 cc. CF3CO2H treated with HBr 90 min. at 0°, the solution evaporated in vacuo, and the residue triturated with Et2O gave 0.95 g. amorphous Pro-Ser-Phe-Gly-Pro-Phe-(O2N)Arg-OMe.1.5HBr.0.5H2O (XIII.HBr), [α]22D -43.6° (c 0.5, DMF). Z-(O2N)Arg-Pro.THF (0.523 g.) in 3 cc. DMF combined at -10° with XIII (from 1 millimole XIII.HBr and Et3N) in 4 cc. DMF and with 0.309 g. DCC in THF and the mixture kept 2 hrs. at 0° and 20 hrs. at room temperature and worked up gave 0.6 g. Z-(O2N)Arg-Pro-Pro-Ser-Phe-Gly-Pro-Phe-(O2N)-Arg-OMe (XIV), [α]22D -59.4° (c 0.5, DMF). XIV (0.455 g.) in 3 cc. dioxane and 1 cc. EtOH stirred 2.5 hrs. with 0.7 cc. N NaOH, the solution evaporated, and the residue dissolved in H2O and acidified with N HCl gave 0.330 g. corresponding benzyloxycarbonylnonapeptide acid, [α]22D -61.8° (c 0.5, DMF), which (270 mg.) hydrogenated in MeOH-AcOH-H2O over Pd black, the filtered solution evaporated in vacuo, and the residue dissolved in H2O and lyophilized gave 250 mg. product, which **purified** by preparative electrophoresis in C5H5N.AcOH buffer (pH 5) gave 131 mg. Arg-Pro-Pro-Ser-Phe-Gly-Pro-Phe-Arg.1.5AcOH.4H2O (Ser4-Gly6-bradykinin) (XV), [α]22D -96.0° (c 0.5, H2O).

From δ-benzylideneornithine and PhCH2O2CCl was prepared 71% Nα-(benzyloxycarbonyl)ornithine (XVI), m. 209-10° (H2O), [α]25D -8.4° (c 1.5, N HCl). XVI (74.5 g.) in 370 cc. H2O and 240 cc. dioxane shaken 4 days at room temperature with 43 g. III and 17 g. MgO in a pressure flask, the solution filtered, concentrated in vacuo to .apprx.1/4 its volume, diluted with H2O, and acidified to pH 2-3 with 20% citric acid, and the oily product (101 g.) **isolated** with EtOAc and recrystd. from EtOAc-petr. ether gave 70 g. Nα-(benzyloxycarbonyl)-Nδ-(tert-butoxycarbonyl)ornithine (XVII), m. 97-8°, [α]25D -8.8° (c 1, C5H5N); dicyclohexylammonium salt m. 128-9° (EtOAc), [α]25D 7.8° (c 1, EtOH). XVII (68 g.) in Et2O-MeOH added dropwise to 900 cc. Et2O-CH2N2 (from 72 g. H2NCONMeNO) at 0° with stirring and the solution kept overnight and worked up gave 63 g. XVII Me ester, m. 70-1° (Et2O-petr. ether), [α]25D -13.3° (c 1, MeOH), which (3.0 g.) hydrogenated in 80 cc. MeOH over Pd black, the filtered solution concentrated, and the residual oily ester treated in EtOAc-Et2O with Et2O-HCl at -20° gave 1.5 g. Nδ-(tert-butoxycarbonyl)ornithine Me ester-HCl (XVIII.HCl), m. 154-5° (MeOH-Et2O-petr. ether), [α]25D 15.5° (c 1, MeOH).

Z-Pro-Phe-NHNH2 (15.0 g.) in 35 cc. 2.2N THF-HCl treated with 4.5 cc. tert-BuONO at -25° with stirring and the solution stirred 10 min. at -25°, diluted with 400 cc. cold (-25°) EtOAc, washed with ice cold saturated aqueous NaHCO3 and H2O, dried, treated with a cold solution of XVIII (from 11.3 g. XVIII.HCl and 5.7 cc. Et3N in CHCl3) at .apprx.-20°, kept 24 hrs. at 0° and 24 hrs. at room temperature, and worked up gave 17.7 g. Z-Pro-Phe-(Nδ-BOC)-Orn-OMe(XIX), m. 120-2° (EtOAc-petr. ether), [α]25D -52.8° (c 1, MeOH), which (12.5 g.) hydrogenated in 200 cc. MeOH over Pd black and the filtered solution concentrated gave 9.1 g. Pro-Phe-(Nδ-BOC)-Orn-OMe (XX), foam, [α]25D -25.6° (c 1, MeOH). Z-Phe-Ser-NHNH2 (6.8 g.) in 20 cc. DMF and 16.1 cc. 2.2N THF-HCl treated like XIX with 2.1 cc. tert-BuONO and the EtOAc solution of the azide treated like XIX with XX (in this case concentration of the EtOAc phase gave crystalline product) yielded 9.4 g.

Z-Phe-Ser-Pro-Phe-(Nδ-BOC)-Orn-OMe, m. 162-4°
(EtOAc-EtOH-petr. ether, EtOH), [α]25D -43.1° (c 1, AcOH),
which (8.6 g.) hydrogenated in 100 cc. MeOH over Pd black gave 4.2 g.
Phe-Ser-Pro-Phe-(Nδ-BOC)-Orn-OMe (XXI), m. 152-3°
(EtOAc-petr. ether), [α]25D -58.3° (c 0.5, MeOH). XIa.HCl
(1.64 g.) in 2 cc. DMF treated at -20° with 2.4 cc. 2.2N THF-HCl
and 0.31 cc. tert-BuONO and after 5-10 min. the solution diluted with 5 cc.
DMF, neutralized with Et3N, filtered, combined with XXI in 3 cc. DMF, kept
24 hrs. at 0° and 24 hrs. at room temperature, and worked up gave 1.6 g.
Z-(O2N)-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-(Nδ-BOC)-Orn-OMe (XXII),
[α]22D -52.4° (c 0.5, DMF). XXII (1.31 g.) in 10 cc. dioxane
stirred 2.5 hrs. with 2 cc. N NaOH, concentrated, diluted with H2O, and
acidified
with 10% aqueous citric acid at 0° gave 1.0 g. corresponding protected
nonapeptide acid, [α]23D -51.4° (c 0.5, DMF), which
(984 mg.) hydrogenated in MeOH-AcOH-H2O over Pd black, the filtered solution
concentrated in vacuo, the residue treated 75 min. with 3 cc. CF3CO2H, and the
solution diluted with cold Et2O gave 0.95 g. product, which (540 mg.)
purified by preparative electrophoresis gave 314 mg.
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Orn.2AcOH.5H2O (Orn9-bradykinin) (XXIII),
[α]22D -86.0° (c 0.5, H2O). The biol. properties of the
bradykinin analogs (discussed) were considerably below the biol. activity
of bradykinin.

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ACCESSION NUMBER: 1959:111411 CAPLUS
DOCUMENT NUMBER: 53:111411
ORIGINAL REFERENCE NO.: 53:19914h-i,19915a-i,19916a-b
TITLE: An improved synthesis of oxytocin
AUTHOR(S): Bodanszky, Miklos; du Vigneaud, Vincent
CORPORATE SOURCE: Cornell Univ. Med. Coll., New York, NY
SOURCE: Journal of the American Chemical Society (1959), 81,
2504-7
CODEN: JACSAT; ISSN: 0002-7863
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
OTHER SOURCE(S): CASREACT 53:111411
AB cf. C.A. 49, 6111b, 12363b; 52, 6188f. A new approach to synthetic
oxytocin is described. By coupling S-benzyl-N carbobenzyloxy-L-cysteinyl-
L-tyrosine (I) with L-isoleucyl-L-glutaminyl-L-asparagine (II) and by the
subsequent condensation of the resulting protected pentapeptide,
S-benzyl-N-carbobenzyloxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-
asparagine (III), with S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide
(IV), the protected **nonapeptide**, S-benzyl-N-carbobenzyloxy-L-
cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-Lasparaginyl-S-benzyl-L-
cysteinyl-L-prolyl-L-leucylglycinamide (V), was prepared After removal of
the protective groups from the **nonapeptide** and oxidation of the
nonapeptide to the disulfide, the biol. active material was
isolated and **purified** by countercurrent distribution.
S-benzyl-N-carbobenzyloxy-L-cysteine (VI) (7 g.) in 22 mL. THF treated
with 4g. p-O2NC6H4OH, 4.2 g. dicyclohexylcarbodiimide added, the solution
allowed to stand about 0.5 h. at room temperature, the precipitate filtered
off, washed
with 22 mL. THF, the THF removed, the residue triturated with 50 mL. 0.2N
KHCO2, filtered, washied with 50 mL. 0.2N KHCO3, 400 mL. H2O, and 100 mL.
90% EtOH yielded 8.2 g. p-nitrophenyl S-benzyl-N-carbobenzyloxy-L-
cysteinate (VII), m 93-4°, [a]22D -42° (c 1, HCONMe2);
[a]23D -37° (c 1, MeOH). VI. (140 g.) and 80 g. Me L-tyrosinate
(VIII) in 200 mL. HCONMe2 treated with 94 g. dicyclohexylcarbodiimide at
0° in 4 portions, the mixture diluted the following day with 650 mL.

EtOAc, filtered, the filtrate and washings extracted with 800 mL. N HCl, 800 mL. H2O, and 0.25% KHCO3 and H2O, dried, evaporated to about 300 mL., dild, with 1250 mL. **hexane**, the Me ester (214.5 g.), m. 92-4°, filtered, a 20% aliquot dissolved in 160 mL. MeOH, treated with 200 mL. N NaOH (cooling), allowed to stand 1 h. at room temperature, filtered with C, and acidified with N HCl 40 min. later, yielded 32 g. I, m. 200-2° [a]23D -14.8 (c 4.3, pyridine). VII. (11.7g.) and 4.4 g. VIII dissolved in 12.5 mL. HCONMe2 the mixture held 3 days, diluted with EtOAc, and treated as I above yielded 9.9 g. I, m. 200-1°, [a]23D -14.8°(c 4.42, pyridine). Cyanomethyl S-benzyl-N-carbobenzyloxy-L-cysteinate (30.7 g.) and 17.5 g. Tosyl-L-isoleucyl-L-glutaminyl-L-asparagine (38 g.) in about 3 l. liquid NH3 treated with 11 g. Na, 30 mL. AcOH added to dissolve precipitate, the NH3 allowed to evaporate (last traces removed in vacuo), the residue in 330 mL. H2O treated with 160 mL. AcOH, the solution filtered with C, the filtrate diluted with 3.5 l. EtOH, allowed to stand 2 days, and filtered yielded 23 g. II, sintered at 225°, decompose 230-5°, [α]24D -32.5° (c 1, 0.5N KHCO3). I (16.8 g.) in 330 mL. THF treated with 5.5. ml. Et3N, 4.5 g. ClCO2-Bu-iso added at -40°, the mixture allowed to warm to -10°, cooled to -40°, 14.2 g. II and 5.5 mL. Et3N in 66 mL. H2O added, the mixture allowed to warm to room temperature, held 4.5 h., diluted with 150 mL. H2O and 75 mL. N HCl, the precipitate (25 g.) filtered off, boiled with 100 mL. EtOAc, filtered off, washed with EtOAc, dissolved in aqueous THF and the solution allowed to evaporate yielded 21.2 g. III, m. 245-7° (decomposition), [α]24D -23.5°. VII (94 g.) and 58.6 g. L-prolyl-L-leucylglycinamide dissolved in 100 mL. HCONMe2, the mixture stirred 2.5 h., allowed to stand 60 h., diluted with 60 mL. EtOAc, refrigerated overnight, and the crystals filtered off yielded 100.5 g. S-benzyl-N-carbobenzyloxy-L-cysteinyl-L-prolyl-L-leucylglycinamide, m. 170-1°, [α]21D -60.0° (c 2, HCONMe2). III (1.73 g.) and 1.1 g. IV in 10 mL. HCONMe2 treated with 0.83 g. dicyclohexylcarbodiimide (cooling), the mixture allowed to come to room temperature, held at room temperature a few hrs., refrigerated overnight, diluted with 1 mL. AcOH and 90 mL. H2O (cooling), the precipitate filtered off (yield 1.85 g., m. 230-8°), 1 g. dissolved in 15 mL. HCONMe2, filtered, washed with 10 mL. HCONMe2, the filtrate treated with 2 drops AcOH and 200 mL. H2O (cooling), the precipitate filtered off, washed with H2O, dried, and the **dry** solid extracted with hot MeOH yielded 0.50 g. S-benzyl-N-carbobenzyloxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IX), m. 243-5° (decompose 247°) [α]22D -43° (c 2, HCONMe2). III (17.26 g.) in 200 mL. HCONMe2 treated with 3 mL. Et3N and 150 mL. THF the mixture, cooled to about -30°, treated with 2.8 g. ClCO2Bu-iso, allowed to warm to -5° during 15 min., cooled to -20°, treated with 11 g. IV.1.5H2O, allowed to warm to room temperature, stirred 1 h., poured into 3 mL. H2O, adjusted to about pH 5 with a few ml. AcOH, and the precipitate filtered off yielded 23 g. IX, m. 220-30° (decomposition). IX (3 g.) extracted with 250 mL. warm MeOH left 0.65 g. material m. 220-35° (decomposition). III (8.7 g.) and 5.5 g. IV dissolved in 50 mL. HCONMe2, 5.2 mL. Et3N and 3.75 g. o-phenylene chlorophosphite added at about -10°, the mixture allowed to come to room temperature, held until the next day, diluted with 600 mL. H2O, 1 mL. AcOH added (pH about 6), and the precipitate separated yielded 12.2 g. IX, m. 223-7° (decomposition); 6.1 g. IX in 150 mL. MeOH held 1 h. at room temperature, filtered, and washed with 150 mL. MeOH yielded 4.8 g., m. 235-6°. IX (1.34 g.) in 450 mL. NH3

treated with 0.35 g. Na, 0.82 g. NH4Cl added, the NH3 allowed to evaporate (the last 30-40 mL. were removed in vacuo), the residue dissolved in 500 mL. H2O, the solution adjusted to pH 6.5 with AcOH, aerated about 4 h., adjusted to about pH 4 with AcOH, filtered, evaporated in vacuo below room temperature to a small volume, dried frozen, the solid from 2 runs extracted with 60 mL. EtOH portionwise, filtered, the filtrate diluted with 500 mL. EtOAc, the precipitate filtered off, washed with EtOAc, dried in vacuo over CaCl2, extracted with 50 mL. pyridine in several portions, filtered, the filtrate diluted with 500 mL. EtOAc, and the precipitate filtered off yielded 1 g. material with approx. 300,000 units.

AB cf. C.A. 49, 6111b, 12363b; 52, 6188f. A new approach to synthetic oxytocin is described. By coupling S-benzyl-N carbobenzyloxy-L-cysteinyl-L-tyrosine (I) with L-isoleucyl-L-glutaminyl-L-asparagine (II) and by the subsequent condensation of the resulting protected pentapeptide, S-benzyl-N-carbobenzyloxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparagine (III), with S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IV), the protected **nonapeptide**, S-benzyl-N-carbobenzyloxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-Lasparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (V), was prepared After removal of the protective groups from the **nonapeptide** and oxidation of the **nonapeptide** to the disulfide, the biol. active material was **isolated** and **purified** by countercurrent distribution. S-benzyl-N-carbobenzyloxy-L-cysteine (VI) (7 g.) in 22 mL. THF treated with 4g. p-O2NC6H4OH, 4.2 g. dicyclohexylcarbodiimide added, the solution allowed to stand about 0.5 h. at room temperature, the precipitate filtered off, washed with 22 mL. THF, the THF removed, the residue triturated with 50 mL. 0.2N KHCO2, filtered, washied with 50 mL. 0.2N KHCO3, 400 mL. H2O, and 100 mL. 90% EtOH yielded 8.2 g. p-nitrophenyl S-benzyl-N-carbobenzyloxy-L-cysteinate (VII), m 93-4°, [a]22D -42° (c 1, HCONMe2); [a]23D -37° (c 1, MeOH). VI. (140 g.) and 80 g. Me L-tyrosinate (VIII) in 200 mL. HCONMe2 treated with 94 g. dicyclohexylcarbodiimide at 0° in 4 portions, the mixture diluted the following day with 650 mL. EtOAc, filtered, the filtrate and washings extracted with 800 mL. N HCl, 800 mL. H2O, and 0.25% KHCO3 and H2O, dried, evaporated to about 300 mL., dild, with 1250 mL. **hexane**, the Me ester (214.5 g.), m. 92-4°, filtered, a 20% aliquot dissolved in 160 mL. MeOH, treated with 200 mL. N NaOH (cooling), allowed to stand 1 h. at room temperature, filtered with C, and acidified with N HCl 40 min. later, yielded 32 g. I, m. 200-2° [a]23D -14.8 (c 4.3, pyridine). VII. (11.7g.) and 4.4 g. VIII dissolved in 12.5 mL. HCONMe2 the mixture held 3 days, diluted with EtOAc, and treated as I above yielded 9.9 g. I, m. 200-1°, [a]23D -14.8°(c 4.42, pyridine). Cyanomethyl S-benzyl-N-carbobenzyloxy-L-cysteinate (30.7 g.) and 17.5 g. Tosyl-L-isoleucyl-L-glutaminyl-L-asparagine (38 g.) in about 3 l. liquid NH3 treated with 11 g. Na, 30 mL. AcOH added to dissolve precipitate, the NH3 allowed to evaporate (last traces removed in vacuo), the residue in 330 mL. H2O treated with 160 mL. AcOH, the solution filtered with C, the filtrate diluted with 3.5 l. EtOH, allowed to stand 2 days, and filtered yielded 23 g. II, sintered at 225°, decompose 230-5°, [α]24D -32.5° (c 1, 0.5N KHCO3). I (16.8 g.) in 330 mL. THF treated with 5.5. ml. Et3N, 4.5 g. ClCO2-Bu-iso added at -40°, the mixture allowed to warm to -10°, cooled to -40°, 14.2 g. II and 5.5 mL. Et3N in 66 mL. H2O added, the mixture allowed to warm to room temperature, held 4.5 h., diluted with 150 mL. H2O and 75 mL. N HCl, the precipitate (25 g.) filtered off, boiled with 100 mL. EtOAc, filtered off, washed with EtOAc, dissolved in aqueous THF and the solution allowed to evaporate yielded 21.2 g.

III, m. 245-7° (decomposition), [α]24D -23.5°. VII (94 g.) and 58.6 g. L-prolyl-L-leucylglycinamide dissolved in 100 mL. HCONMe2, the mixture stirred 2.5 h., allowed to stand 60 h., diluted with 60 mL. EtOAc, refrigerated overnight, and the crystals filtered off yielded 100.5 g. S-benzyl-N-carbobenzyloxy-L-cysteinyl-L-prolyl-L-leucylglycinamide, m. 170-1°, [α]21D -60.0° (c 2, HCONMe2). III (1.73 g.) and 1.1 g. IV in 10 mL. HCONMe2 treated with 0.83 g. dicyclohexylcarbodiimide (cooling), the mixture allowed to come to room temperature, held at room temperature a few hrs., refrigerated overnight, diluted with 1 mL. AcOH and 90 mL. H2O (cooling), the precipitate filtered off (yield 1.85 g., m. 230-8°), 1 g. dissolved in 15 mL. HCONMe2, filtered, washed with 10 mL. HCONMe2, the filtrate treated with 2 drops AcOH and 200 mL. H2O (cooling), the precipitate filtered off, washed with H2O, dried, and the **dry** solid extracted with hot MeOH yielded 0.50 g. S-benzyl-N-carbobenzyloxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IX), m. 243-5° (decompose 247°) [α]22D -43° (c 2, HCONMe2). III (17.26 g.) in 200 mL. HCONMe2 treated with 3 mL. Et3N and 150 mL. THF the mixture, cooled to about -30°, treated with 2.8 g. ClCO2Bu-iso, allowed to warm to -5° during 15 min., cooled to -20°, treated with 11 g. IV.1.5H2O, allowed to warm to room temperature, stirred 1 h., poured into 3 mL. H2O, adjusted to about pH 5 with a few ml. AcOH, and the precipitate filtered off yielded 23 g. IX, m. 220-30° (decomposition). IX (3 g.) extracted with 250 mL. warm MeOH left 0.65 g. material m. 220-35° (decomposition). III (8.7 g.) and 5.5 g. IV dissolved in 50 mL. HCONMe2, 5.2 mL. Et3N and 3.75 g. o-phenylene chlorophosphite added at about -10°, the mixture allowed to come to room temperature, held until the next day, diluted with 600 mL. H2O, 1 mL. AcOH added (pH about 6), and the precipitate separated yielded 12.2 g. IX, m. 223-7° (decomposition); 6.1 g. IX in 150 mL. MeOH held 1 h. at room temperature, filtered, and washed with 150 mL. MeOH yielded 4.8 g., m. 235-6°. IX (1.34 g.) in 450 mL. NH3 treated with 0.35 g. Na, 0.82 g. NH4Cl added, the NH3 allowed to evaporate (the last 30-40 mL. were removed in vacuo), the residue dissolved in 500 mL. H2O, the solution adjusted to pH 6.5 with AcOH, aerated about 4 h., adjusted to about pH 4 with AcOH, filtered, evaporated in vacuo below room temperature to a small volume, dried frozen, the solid from 2 runs extracted with 60 mL. EtOH portionwise, filtered, the filtrate diluted with 500 mL. EtOAc, the precipitate filtered off, washed with EtOAc, dried in vacuo over CaCl2, extracted with 50 mL. pyridine in several portions, filtered, the filtrate diluted with 500 mL. EtOAc, and the precipitate filtered off yielded 1 g. material with approx. 300,000 units.

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ACCESSION NUMBER:	1958:25222 CAPLUS
DOCUMENT NUMBER:	52:25222
ORIGINAL REFERENCE NO.:	52:4498a-g
TITLE:	Synthesis of lysine-vasopressin
AUTHOR(S):	du Vigneaud, Vincent; Bartlett, M. Frederick; Johl, Albert
CORPORATE SOURCE:	Cornell Univ. Med. Coll., New York, NY
SOURCE:	Journal of the American Chemical Society (1957), 79, 5572-5 CODEN: JACSAT; ISSN: 0002-7863
DOCUMENT TYPE:	Journal
LANGUAGE:	Unavailable

Prepared by: Mary Hale @2-2507 Rem Bldg 1D86

OTHER SOURCE(S): CASREACT 52:25222

AB cf. C.A. 50, 14539e. An octapeptide amide with the structure proposed for lysine-vasopressin (I) was synthesized through coupling S-benzyl-N-tosyl-L-cysteinyl-L- tyrosyl-L-phenylalanyl-L-glutamyl-L-asparagine (II) with S-benzyl-L-cysteinyl-L-prolyl-Ne-tosyl-L-lysylglycinamide (III) in the presence of (C6H11N:)2C (IV) to yield the protected **nonapeptide** amide (V), followed by reduction with Na in liquid NH3 and oxidation of the product. II was prepared by coupling S-benzyl-N-tosyl-L-cysteinyl-L-tyrosine (VI) with L-phenylalanyl-L-glutaminyl-L-asparagine (VII) by the mixed anhydride method. The synthetic octapeptide was **purified** by countercurrent distribution and electrophoresis. Comparison of the chemical, phys., and biol. behavior of the synthetic product with that of I **isolated** from hog posterior pituitary glands led to the conclusion that the synthetic product is lysine-vasopressin. S-Benzyl-L-cysteine (20 g.) in 120 cc. 3N NaOH and 450 cc. H2O treated with 32 g. p-MeC6H4SO2Cl in 100 cc. Et2O, the mixture stirred 3 hrs. at room temperature (alkaline reaction maintained by addition of NaOH pellets), the aqueous phase washed with Et2O, slowly acidified with 50% HCl, and filtered yielded 27.0 g. S-benzyl-N-tosyl-L-cysteine (VIII), m. 125-6°, [α]21D 11.3° (c 2.00, EtOH). VIII (11 g.) in 50 cc. tetrahydrofuran cooled with ice H2O, Et L-tyrosinate prepared from 7.5 g. HCl salt and 4.1 cc. Et3N in 50 cc. tetrahydrofuran, and 6.9 g. IV added, the mixture stirred 4 hrs. with cooling, filtered, the filtrate evaporated to **dryness** in vacuo, the residue washed in EtOAc, evaporated to a small volume, and treated dropwise during 2 hrs. with **hexane** yielded 14.5 g. S-benzyl-N-tosyl-L-cysteinyl-L-tyrosine Et ester (IX), m. 109-10°, [α]19D 3.71° (c 2.39, EtOH). IX (2 g.) in 6 cc. Me2CO (ice cold) treated portionwise with 6 cc. 2N NaOH during 20 min., the mixture held 40 min. at room temperature, diluted with 10 cc. H2O, acidified with concentrated HCl (cooling), the product filtered, dissolved in 5% NaHCO3, extracted with EtOAc, and acidified with concentrated HCl yielded 1.80 g. VI, m. 155-6°, [α]18D 28.2° (c 2.11, absolute EtOH), [α]23D 23.6° (c 2.85, EtOH). VI (2.64 g.) in 25 cc. tetrahydrofuran at -10°, treated with 0.70 cc. Et3N and 0.65 cc. ClCO2CH2CHMe2, the mixture stirred 7 min. at -15°, treated during 1 min. with 2.04 g. VII in 10 cc. H2O containing 0.75 cc. Et3N, cooled to the f.p., the solution stirred 5 min. at -10°, 30 min. at room temperature, diluted to turbidity with H2O, acidified to pH 2 with 50% HCl, made up to 100 cc. with H2O, held 4 hrs. at 0°, and filtered yielded 2.84 g. II, m. 203-5°, [α]21D 4.4° (c 2.08, HCONMe2). II (1.84 g.) and 1.29 g. III in 100 cc. 90% tetrahydrofuran treated (ice cooling) with 0.62 g. IV, the mixture stirred 4 hrs. with cooling, 20 hrs. at room temperature, concentrated to about 20 cc. in vacuo, cooled 1 hr., filtered, the solid extracted with 20 cc. HCONMe2, filtered, the filtrate diluted with 80 cc. EtOAc, cooled overnight, and filtered yielded 1.21 g. V, m. 226-30°, [α]18D -23.0° (c 2.11, HCONMe2).

AB cf. C.A. 50, 14539e. An octapeptide amide with the structure proposed for lysine-vasopressin (I) was synthesized through coupling S-benzyl-N-tosyl-L-cysteinyl-L- tyrosyl-L-phenylalanyl-L-glutamyl-L-asparagine (II) with S-benzyl-L-cysteinyl-L-prolyl-Ne-tosyl-L-lysylglycinamide (III) in the presence of (C6H11N:)2C (IV) to yield the protected **nonapeptide** amide (V), followed by reduction with Na in liquid NH3 and oxidation of the product. II was prepared by coupling S-benzyl-N-tosyl-L-cysteinyl-L-tyrosine (VI) with L-phenylalanyl-L-glutaminyl-L-asparagine (VII) by the mixed anhydride method. The synthetic octapeptide was **purified** by countercurrent

distribution and electrophoresis. Comparison of the chemical, phys., and biol. behavior of the synthetic product with that of I **isolated** from hog posterior pituitary glands led to the conclusion that the synthetic product is lysine-vasopressin. S-Benzyl-L-cysteine (20 g.) in 120 cc. 3N NaOH and 450 cc. H2O treated with 32 g. p-MeC6H4SO2Cl in 100 cc. Et2O, the mixture stirred 3 hrs. at room temperature (alkaline reaction maintained by addition of NaOH pellets), the aqueous phase washed with Et2O, slowly acidified with 50% HCl, and filtered yielded 27.0 g. S-benzyl-N-tosyl-L-cysteine (VIII), m. 125-6°, [α]21D 11.3° (c 2.00, EtOH). VIII (11 g.) in 50 cc. tetrahydrofuran cooled with ice H2O, Et L-tyrosinate prepared from 7.5 g. HCl salt and 4.1 cc. Et3N in 50 cc. tetrahydrofuran, and 6.9 g. IV added, the mixture stirred 4 hrs. with cooling, filtered, the filtrate evaporated to **dryness** in vacuo, the residue washed in EtOAc, evaporated to a small volume, and treated dropwise during 2 hrs. with **hexane** yielded 14.5 g. S-benzyl-N-tosyl-L-cysteinyl-L-tyrosine Et ester (IX), m. 109-10°, [α]19D 3.71° (c 2.39, EtOH). IX (2 g.) in 6 cc. Me2CO (ice cold) treated portionwise with 6 cc. 2N NaOH during 20 min., the mixture held 40 min. at room temperature, diluted with 10 cc. H2O, acidified with concentrated HCl (cooling), the product filtered, dissolved in 5% NaHCO3, extracted with EtOAc, and acidified with concentrated HCl yielded 1.80 g. VI, m. 155-6°, [α]18D 28.2° (c 2.11, absolute EtOH), [α]23D 23.6° (c 2.85, EtOH). VI (2.64 g.) in 25 cc. tetrahydrofuran at -10°, treated with 0.70 cc. Et3N and 0.65 cc. ClCO2CH2CHMe2, the mixture stirred 7 min. at -15°, treated during 1 min. with 2.04 g. VII in 10 cc. H2O containing 0.75 cc. Et3N, cooled to the f.p., the solution stirred 5 min. at -10°, 30 min. at room temperature, diluted to turbidity with H2O, acidified to pH 2 with 50% HCl, made up to 100 cc. with H2O, held 4 hrs. at 0°, and filtered yielded 2.84 g. II, m. 203-5°, [α]21D 4.4° (c 2.08, HCONMe2). II (1.84 g.) and 1.29 g. III in 100 cc. 90% tetrahydrofuran treated (ice cooling) with 0.62 g. IV, the mixture stirred 4 hrs. with cooling, 20 hrs. at room temperature, concentrated to about 20 cc. in vacuo, cooled 1 hr., filtered, the solid extracted with 20 cc. HCONMe2, filtered, the filtrate diluted with 80 cc. EtOAc, cooled overnight, and filtered yielded 1.21 g. V, m. 226-30°, [α]18D -23.0° (c 2.11, HCONMe2).

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L55          13 FILE MEDLINE
L56           9 FILE BIOSIS
L57           6 FILE EMBASE
L58          15 FILE CAPLUS

TOTAL FOR ALL FILES
L59          43 L49 NOT L54


=> dup rem l59
PROCESSING COMPLETED FOR L59
L60           28 DUP REM L59 (15 DUPLICATES REMOVED)

=> d 1-28 ibib abs

L60  ANSWER 1 OF 28  EMBASE  COPYRIGHT (c) 2005 Elsevier B.V. All rights
     reserved on STN
ACCESSION NUMBER:   2004337982  EMBASE


Prepared by: Mary Hale @2-2507 Rem Bldg 1D86
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TITLE: How we drifted into peptide chemistry and where we have arrived atomic
AUTHOR: Seebach D.; Kimmerlin T.; Sebesta R.; Campo M.A.; Beck A.K.
CORPORATE SOURCE: D. Seebach, Dept. F. Chem. Angew. B., ETH Honggerberg, Wolfgang-Pauli-Strasse 10, CH-8093, Zurich, Switzerland. seebach@org.chem.ethz.ch
SOURCE: Tetrahedron, (23 Aug 2004) Vol. 60, No. 35, pp. 7455-7506. Refs: 853
ISSN: 0040-4020 CODEN: TETRAB
PUBLISHER IDENT.: S 0040-4020(04)00911-1
COUNTRY: United Kingdom
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
037 Drug Literature Index
039 Pharmacy
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 20040826
Last Updated on STN: 20040826

AB The history of peptide chemistry in our group is described. It all started with the cyclic undecapeptide cyclosporin, the immunosuppressive compound, which is commercialised as Sandimmune(®)/Neoral(®) by Sandoz/Novartis, and which has revolutionized transplant medicine. The discovery that cyclosporin can be deprotonated to a hexalithio derivative, and thus C-alkylated on a sarcosine moiety, led us into a research project on peptide modifications. We defined structural prerequisites for the use of peptide enolates and for electrolytic decarboxylation of peptides. Parallel to these activities, the group was engaged in developing synthetic methodologies aimed at stereoselective preparations of α-, β-, and γ-amino acid derivatives (cf. diastereoselective alkylations, self regeneration of stereogenic centers, axially chiral enolates). A third avenue into peptide chemistry originated from our investigations on the biopolymer PHB (poly-3-hydroxybutanoic acid); the question arose 'what happens upon replacement of chain-bound O by NH in the polyester?' A brief summary is given of the results obtained in our ensuing discovery tour of β-peptides built of homologated proteinogenic amino acids. They form secondary structures with short chain lengths and they have unexpected physiological properties, rendering them candidates for peptidic drugs. The synthesis of β(3)-peptides is straightforward, and in the meantime most of the Fmoc-protected building blocks are commercial. The β(2)-homoamino acids are less readily available. Their preparation and the assembly of a β(2)-eicosapeptide with the twenty proteinogenic side chains are discussed herein. The reasons for the chosen sequence and the strategy of what turned out to be a 159-step synthesis are described. Full experimental details are given for the preparation of the dimeric Fmoc-β(2)hXaa(PG)- β(2)hXaa(PG)-OH building blocks used, for their solid-phase coupling to two β(2)- **decapeptide** segments, for the thioligation, and for the **purification**, isolation and spectroscopic characterization of the resulting 20mer. An outlook to future projects in the exciting field of β- and γ-peptide chemistry and biology is given. .COPYRGT. 2004 Elsevier Ltd. All rights reserved.

L60 ANSWER 2 OF 28 MEDLINE on STN DUPLICATE 1
ACCESSION NUMBER: 2003527488 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14604374
TITLE: Chemical synthesis of CD52 glycopeptides containing the acid-labile fucosyl linkage.
AUTHOR: Shao Ning; Xue Jie; Guo Zhongwu

CORPORATE SOURCE: Department of Chemistry, Case Western Reserve University, Cleveland, OH 44106, USA.
SOURCE: Journal of organic chemistry, (2003 Nov 14) 68 (23) 9003-11.
Journal code: 2985193R. ISSN: 0022-3263.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200403
ENTRY DATE: Entered STN: 20031108
Last Updated on STN: 20040327
Entered Medline: 20040326

AB Glycopeptide 1 with the fucosylated trisaccharide, beta-d-GlcNAc(1-->4)[alpha-l-Fuc(1-->6)]-beta-d-GlcNAc, linked to the Asn of CD52 peptide was prepared by two methods, both of which used the free glycosyl Asn 12 and glycotripeptide 21 as key intermediates. Thus, after the trisaccharide was prepared and linked to Asn, the carbohydrate moiety was deprotected to give 12. From 12, 21 was constructed in homogeneous NMP solutions by elongating the peptide chain alone the N-terminus. Though the glycopeptides were easily soluble in NMP, they were barely soluble in diethyl ether, because of the free trisaccharide. Consequently, addition of diethyl ether to the reaction mixtures could precipitate the glycopeptides, and the products were conveniently isolated and **purified** in the solid form. The coupling of 21 with a free **nonapeptide** 24 in NMP afforded 1. 1 was also prepared by solid-phase synthesis, using the acid-sensitive 2-chlorotrityl resin. In this case, 21 was attached to the **nonapeptide** on the resin, and the resulting glycopeptide was then released with dilute **acetic acid.** Deprotection of the peptide under moderate acidic conditions gave 1. The acid-labile alpha-fucose was not affected in these syntheses.

L60 ANSWER 3 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 2000:67423 CAPLUS
DOCUMENT NUMBER: 132:119544
TITLE: Matrices with memories and uses thereof
INVENTOR(S): Nova, Michael P.; Parandoosh, Zahra; Senyei, Andrew E.; Xiao, Xiao-Yi; David, Gary S.; Satoda, Yozo; Zhao, Chanfeng; Potash, Hanan
PATENT ASSIGNEE(S): Irori, USA
SOURCE: U.S., 113 pp., Cont.-in-part of U.S. Ser. No. 711,426.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 20
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6017496	A	20000125	US 1996-709435	19960906
US 5741462	A	19980421	US 1995-428662	19950425
US 5925562	A	19990720	US 1995-480196	19950607
US 6331273	B1	20011218	US 1995-473660	19950607
US 6352854	B1	20020305	US 1995-480147	19950607
US 6416714	B1	20020709	US 1995-484486	19950607
US 5874214	A	19990223	US 1995-538387	19951003
US 6025129	A	20000215	US 1995-567746	19951205
WO 9636436	A1	19961121	WO 1996-US6145	19960425

W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE,

ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN

US 6319668	B1	20011120	US 1996-669252		19960624
US 6284459	B1	20010904	US 1996-711426		19960905
US 5961923	A	19991005	US 1996-723423		19960930
WO 9712680	A2	19970410	WO 1996-US15999		19961003
WO 9712680	A3	19970821			

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG

AU 9672573	A1	19970428	AU 1996-72573		19961003
EP 853497	A2	19980722	EP 1996-934064		19961003

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI

WO 9749653	A2	19971231	WO 1997-US11035		19970624
WO 9749653	A3	19980226			

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RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

AU 9735779	A1	19980114	AU 1997-35779		19970624
US 6329139	B1	20011211	US 1997-912998		19970811
US 6340588	B1	20020122	US 1998-51022		19980922

PRIORITY APPLN. INFO.:

US 1995-428662	A2	19950425
US 1995-184504	A2	19950607
US 1995-473660	A2	19950607
US 1995-480147	A2	19950607
US 1995-480196	A2	19950607
US 1995-484486	A2	19950607
US 1995-538387	A2	19951003
US 1995-567746	A2	19951205
US 1996-639813	B2	19960402
WO 1996-US6145	A2	19960425
US 1996-669252	A2	19960624
US 1996-711426	A2	19960905
US 1995-484504	A2	19950607
US 1996-633410	A2	19960610
US 1996-20706P	P	19960624
US 1996-709435	A2	19960906
US 1996-723423	A	19960930
WO 1996-US15999	W	19961003
US 1996-726703	B2	19961007
US 1996-743984	A2	19961028
US 1996-741685	B2	19961031
US 1997-857800	B2	19970122
US 1997-826253	B2	19970327
WO 1997-US11035	W	19970624
US 1997-945053	B2	19971021

AB Combinations, called matrixes with memories, of matrix materials that are

encoded with an optically readable code are provided. The matrix materials are those that are used in as supports in solid phase chemical and biochem. syntheses, immunoassays and hybridization reactions. The matrix materials may addnl. include fluorophors or other luminescent moieties to produce luminescing matrixes with memories. The memories include electronic and optical storage media and also include optical memories, such as bar codes and other machine-readable codes. By virtue of this combination, mols. and biol. particles, such as phage and viral particles and cells, that are in proximity or in phys. contact with the matrix combination can be labeled by programming the memory with identifying information and can be identified by retrieving the stored information. Combinations of matrix materials, memories, and linked mols. and biol. materials are also provided. The combinations have a multiplicity of applications, including combinatorial chemical, isolation and **purifn** . of target macromols., capture and detection of macromols. for anal. purposes, selective removal of contaminants, enzymic catalysis, cell sorting, drug delivery, chemical modification and other uses. Methods for tagging mols., biol. particles and matrix support materials, immunoassays, receptor binding assays, scintillation proximity assays, non-radioactive proximity assays, and other methods are also provided.
Scintillant-encased glass beads and chips were prepared and used in assays.

REFERENCE COUNT: 719 THERE ARE 719 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L60 ANSWER 4 OF 28 MEDLINE on STN DUPLICATE 2
ACCESSION NUMBER: 2000475889 MEDLINE
DOCUMENT NUMBER: PubMed ID: 10964719
TITLE: A novel avian hypothalamic peptide inhibiting gonadotropin release.
AUTHOR: Tsutsui K; Saigoh E; Ukena K; Teranishi H; Fujisawa Y; Kikuchi M; Ishii S; Sharp P J
CORPORATE SOURCE: Laboratory of Brain Science, Hiroshima University, Higashi-Hiroshima, 739-8521, Japan.. tsutsui@hiroshima-u.ac.jp
SOURCE: Biochemical and biophysical research communications, (2000 Aug 28) 275 (2) 661-7.
Journal code: 0372516. ISSN: 0006-291X.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200010
ENTRY DATE: Entered STN: 20001012
Last Updated on STN: 20001012
Entered Medline: 20001003

AB The neuropeptide control of gonadotropin secretion at the level of the anterior pituitary gland is primarily through the stimulatory action of the hypothalamic **decapeptide**, gonadotropin-releasing hormone (GnRH), which was originally isolated from mammals and subsequently from non-mammals. To date, however, an inhibitory peptide of gonadotropin release is unknown in vertebrates. Here we show, in a bird, that the hypothalamus also contains a novel peptide which inhibits gonadotropin release. **Acetic acid** extracts of quail brains were passed through C-18 reversed-phase cartridges, and then the retained material was subjected to the reversed-phase and cation-exchange high-performance liquid chromatography (HPLC). The peptide was isolated from avian brain and shown to have the sequence Ser-Ile-Lys-Pro-Ser-Ala-Tyr-Leu-Pro-Leu-Arg-Phe-NH(2). Cell bodies and terminals containing this peptide were localized immunohistochemically in the paraventricular

nucleus and median eminence, respectively. This peptide inhibited, in a dose-related way, gonadotropin release from cultured quail anterior pituitaries. This is the first hypothalamic peptide inhibiting gonadotropin release reported in a vertebrate. We therefore term it gonadotropin-inhibitory hormone (GnIH).

L60 ANSWER 5 OF 28 MEDLINE on STN
ACCESSION NUMBER: 95195295 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7888714
TITLE: High yield, directed immobilization of a peptide-ligand onto a beaded cellulose support.
AUTHOR: Englebretsen D R; Harding D R
CORPORATE SOURCE: Massey University, Palmerston North, New Zealand.
SOURCE: Peptide research, (1994 Nov-Dec) 7 (6) 322-6.
Journal code: 8913494. ISSN: 1040-5704.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199504
ENTRY DATE: Entered STN: 19950427
Last Updated on STN: 19950427
Entered Medline: 19950420

AB Aminopropyl derivatized Perloza beaded cellulose was acylated with alpha-bromoacetic anhydride to give alpha-bromo-acetamidopropyl Perloza. (N-Acetyl)-Cys-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2, the 7 C-terminal amino acids of the **decapeptide** luteinizing hormone-releasing hormone with a cysteine added to the N-terminus, was synthesized using Fmoc chemistry. The **purified** peptide (1.35-1.9 eq) was coupled to alpha-bromoacetamidopropyl Perloza in 0.1 M NaHCO3 solution, pH 8.3, for 1-2 hours. The peptide was anchored to the support via a thioether linkage. Analysis of the peptide-Perloza conjugate indicated near-quantitative displacement of support-bound bromine by the peptide. The peptidic affinity matrix was able to bind ovine antibodies to luteinizing hormone-releasing hormone (LHRH). Thioether immobilization offers directed, chemically stable, high-yield anchoring of synthetic peptides onto a chromatographic support. The high reaction efficiency means there is little waste of valuable synthetic peptide.

L60 ANSWER 6 OF 28 EMBASE
ACCESSION NUMBER: 90143053 EMBASE
DOCUMENT NUMBER: 1990143053
TITLE: Glucosamine-6-phosphate synthase from Escherichia coli: Determination of the mechanism of inactivation by N3-fumaroyl-L-2,3-diaminopropionic derivatives.
AUTHOR: Kucharczyk N.; Denisot M.-A.; Le Goffic F.; Badet B.
CORPORATE SOURCE: Laboratoire de Bioorganique, UA CNRS 1389, ENSCP, 11 Rue Pierre et Marie Curie,75231 Paris Cedex 05, France
SOURCE: Biochemistry, (1990) Vol. 29, No. 15, pp. 3668-3676.
ISSN: 0006-2960 CODEN: BICHAW
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 004 Microbiology
029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
ENTRY DATE: Entered STN: 911213
Last Updated on STN: 911213

Prepared by: Mary Hale @2-2507 Rem Bldg 1D86

AB A mechanistic investigation of the inactivation of Escherichia coli glucosamine-6-phosphate synthase by N3-(4-methoxyfumaroyl)-L-2,3-diaminopropionate (FMDP) was undertaken. On the bases of the known participation of the N-terminal cysteine residue in this process [Chmara et al. (1986) Biochim. Biophys. Acta 870, 357; Badet et al. (1988) Biochemistry 27, 2282], the model reactions between FMDP and L-cysteine and between FMDP and the synthetic **decapeptide** Cys-GLy-Ile-Val-Gly-Ala-Ile-Ala-Gln-Arg, corresponding to the amino-terminal protein sequence, were studied. The results allowed us to propose a pathway that is in perfect agreement with the biochemical results: enzyme inactivation arose from Michael addition of glutamine binding site cysteine-1 on the fumaroyl double bond at the β-position of the ester group. Upon denaturation under slightly alkaline conditions, this adduct underwent cyclization to a transient succinimide adduct, which rearranged into the stable 2-substituted 1,4-thiazin-3-one-5-carboxylate involving participation of the cysteine amino group. The tryptic radiolabeled peptides **purified** from [3H]FMDP-treated enzyme and resistant to Edman degradation coeluted with the products resulting from the model reaction between the synthetic **decapeptide** and the inhibitor.

L60 ANSWER 7 OF 28 MEDLINE on STN
ACCESSION NUMBER: 91177653 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2079391
TITLE: Solution phase synthesis of Saccharomyces cerevisiae a-mating factor and its analogs.
AUTHOR: Xue C B; Ewenson A; Becker J M; Naider F
CORPORATE SOURCE: Department of Chemistry, College of Staten Island, City University of New York.
CONTRACT NUMBER: GM22086 (NIGMS)
GM22087 (NIGMS)
SOURCE: International journal of peptide and protein research, (1990 Oct) 36 (4) 362-73.
Journal code: 0330420. ISSN: 0367-8377.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199104
ENTRY DATE: Entered STN: 19910519
Last Updated on STN: 19910519
Entered Medline: 19910429

AB The solution phase synthesis of the Saccharomyces cerevisiae a-mating factor and nonfarnesylated and nonmethylated a-factor analogs are reported. The a-factor, a lipopeptide with the sequence Tyr-Ile-Ile-Lys-Gly-Val-Phe-Trp-Asp-Pro-Ala-Cys(S-Farnesyl)OCH3 was synthesized by the condensation of the amine terminal protected **decapeptide** with the carboxyl terminal farnesylated dipeptide using benzotriazol-1-yloxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent) as the coupling agent. The synthesis of the **decapeptide** involved 5 + 5 fragment coupling with the BOP reagent and the successful application of 9-fluorenylmethyl ester(OFm) and 9-fluorenylmethoxycarbonyl(Fmoc) groups for the protection of Asp and Lys side chains and Tyr alpha-amine and of phenacyl esters (OPa) for alpha-carboxyl protection. The OFm and Fmoc groups tolerated repeated couplings and were completely stable to zinc powder in **acetic acid**, a condition under which the OPa group was removed. The synthesis of the nonfarnesylated alpha-factor was accomplished by the coupling of the **decapeptide** with tetrapeptide (Ala-CysOCH3)2 followed by the deprotection of the OFm and Fmoc groups with piperidine

and the cleavage of the disulfide bond with zinc powder in **acetic acid.** The nonmethylated a-factor was prepared by 10 + 2 fragment coupling using OFm protection of the dipeptide carboxyl group followed by removal of all protecting groups with piperidine. Attempts to saponify a-factor were not successful. The synthetic nonfarnesylated and nonmethylated a-mating pheromones were 100-1000 times less active than the a-factor, indicating that although the methyl ester and the farnesyl group are not essential for biological activity, they are necessary for high potency.

L60 ANSWER 8 OF 28 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 91107186 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2272747
TITLE: Isolation and structure elucidation of bovine pineal arginine vasopressin: arginine vasotocin not identified.
AUTHOR: Benson B; Ebels I; Hruby V J
CORPORATE SOURCE: Department of Anatomy, University of Arizona, Tucson.
CONTRACT NUMBER: HD 19521 (NICHD)
SOURCE: International journal of peptide and protein research, (1990 Aug) 36 (2) 109-21.
Journal code: 0330420. ISSN: 0367-8377.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199102
ENTRY DATE: Entered STN: 19910329
Last Updated on STN: 19970203
Entered Medline: 19910228

AB A large number of reports have demonstrated the presence of neurohypophysial hormone-like peptides in mammalian pineal glands and an antigonadotropic function has been ascribed to pineal arginine vasotocin (AVT). We have undertaken large scale **purification** of bovine pineal neurohypophysial hormone-like substances which demonstrate mouse mammary milk-ejection activity (ME-activity) in vitro. Peptides with ME-activity were extracted from more than 5 kg of bovine pineal glands. ME-activity containing peptides were found in both high (Mr approximately 10,000-15,000) and low (Mr approximately 500-1000) Mr species from Sephadex G-25 chromatography of 0.2 N **acetic acid** extracts. After ultrafiltration in 5% formic acid, the neurohypophysial hormone-like peptides were localized to an ultrafiltration Mr 500-1000 retentate. A homogeneous peptide, which shared an identical retention time (RT) and amino acid sequence with synthetic 8-arginine vasopressin (AVP), was isolated by serial semipreparative high performance liquid chromatography. On the other hand, the non-mammalian **nonapeptide** AVT was not identified.

L60 ANSWER 9 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1991:506287 CAPLUS
DOCUMENT NUMBER: 115:106287
TITLE: Isolation and structure elucidation of bovine pineal oxytocin and an antigonadotropic peptide
AUTHOR(S): Benson, Bryant; Ebels, Ietskina; Hruby, Victor J.
CORPORATE SOURCE: Dep. Anat. Chem., Univ. Arizona, Tucson, AZ, 85724, USA
SOURCE: Advances in Pineal Research (1990), 4, 99-111
CODEN: APIREW; ISSN: 0269-0071
DOCUMENT TYPE: Journal
LANGUAGE: English

AB A large number of reports have demonstrated the presence of neurohypophysial

hormone-like peptides in mammalian pineal glands and an antigonadotropic function has been imputed to pineal arginine vasotocin (AVT). The authors recently completed a large-scale preparation and **purification** of pineal neurohypophysial hormone-like peptides which demonstrate mouse mammary milk-ejection activity (ME-activity) in vitro. More than 5 kg of defatted bovine pineals were homogenized and extracted in dilute **acetic acid**, centrifuged and the supernate fractions ultrafiltered. The concentration filtrates <30,000 Mr, containing both neurophysin-bound and free **nonapeptides**, were chromatographed on Sephadex G-25 cdolumns in 0.2N acetic aci. After further ultrafiltration in formic acid and dissociation from carrier proteins, eluted peptides with ME-activity were **purified** by serial semi-preparative HPLC on C-8 300 and 60 Å columns with binary and ternary gradient mobile phases. Single 210 nm absorbance peaks were identified which co-eluted with synthetic arginine vasopressin (AVP) but not with the putative pineal antigonadotropic hormone arginine vasotocin (AVT). The amino acid and automated microsequence analyses of isolated bovine pineal 8-arginine vasopressin were reported elsewhere. The isolation and identification of pineal oxytocin (OT) is reported herein. Hydrolysis of a highly **purified** preparation of bovine OT-like peptide yielded the primary amino acids of OT in ratios highly consistent with theor. amts. Automated microsequence anal. indicated the primary structure of pineal OT to be identical with a synthetic OT standard During the final **purification** of pineal OT, a peptide was identified that increased anterior pituitary dopamine content in mice. Primary amino acid anal. revealed a novel, cysteine-free **decapeptide** rich in threonine.

L60 ANSWER 10 OF 28 MEDLINE on STN DUPLICATE 4
ACCESSION NUMBER: 89388832 MEDLINE
DOCUMENT NUMBER: PubMed ID: 2781578
TITLE: High resolution of honey bee (Apis mellifera) venom peptides by **propionic acid**/urea polyacrylamide gel electrophoresis after ethanol precipitation.
AUTHOR: Chettibi S; Lawrence A
CORPORATE SOURCE: Department of Cell Biology, University of Glasgow, Scotland.
SOURCE: Toxicon : official journal of the International Society on Toxinology, (1989) 27 (7) 781-7.
Journal code: 1307333. ISSN: 0041-0101.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198910
ENTRY DATE: Entered STN: 19900309
Last Updated on STN: 19980206
Entered Medline: 19891020

AB A new and simple gel electrophoretic method is described which enables the protein and polypeptide components of bee venom to be resolved on a single gel. The electrophoretic method allows octapeptides to be resolved and species as small as **decapeptides** can be detected at high sensitivity using the Coomassie blue staining method without prior fixation. This has been achieved by replacing **acetic acid** by **propionic acid** in acid/urea polyacrylamide gels and by controlling the amount of TEMED catalyst for the polymerisation of high concentration gels in order to obtain a low effective pore size. We demonstrated the value of ethanol precipitation as a rapid and efficient desalting the fractionation technique and propose that it could be used in combination with gel filtration to **purify**

many of the peptides to homogeneity.

L60 ANSWER 11 OF 28 MEDLINE on STN
ACCESSION NUMBER: 86273706 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3015516
TITLE: Raising antibodies by coupling peptides to PPD and immunizing BCG-sensitized animals.
AUTHOR: Lachmann P J; Strangeways L; Vyakarnam A; Evan G
SOURCE: Ciba Foundation symposium, (1986) 119 25-57. Ref: 53
Journal code: 0356636. ISSN: 0300-5208.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198609
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 19900321
Entered Medline: 19860916

AB The use of PPD (**purified** protein derivative of tuberculin) as a carrier has several significant advantages. It provides very powerful T cell help and it gives rise to virtually no antibody response against itself. This is particularly useful if it is intended to go on to make monoclonal antibodies, where the presence of a large amount of anti-carrier antibody is a nuisance! Furthermore, unlike most comparably powerful adjuvant systems, it can be used in man. PPD coupling has been used to raise antibodies to haptens and to raise T cell responses to tumour cells. It is here reported that small peptides coupled to PPD will give rise to good titres of anti-peptide antibody. For peptides that contain no cysteine, coupling has been achieved by attaching succinimidyl 4-(N-maleimidomethyl) **cyclohexane**-1-carboxylate (SMCC) to the alpha-amino group of the peptide and N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) to the PPD and allowing an uncleavable bond to form between them. Data on immunization with the leucotactic **nonapeptide** of the alpha chain of the complement component C3 and with some oncogene-related peptides have been obtained.

L60 ANSWER 12 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1986:162598 CAPLUS
DOCUMENT NUMBER: 104:162598
TITLE: **Decapeptide** having gonadotropin releasing activity
INVENTOR(S): Miyamoto, Kaoru
PATENT ASSIGNEE(S): Kanegafuchi Chemical Industry Co., Ltd. , Japan
SOURCE: U.S., 3 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 4540513	A	19850910	US 1984-654289	19840925
CA 1251900	A1	19890328	CA 1984-463622	19840919

PRIORITY APPLN. INFO.: US 1984-654289 19840925

AB A **decapeptide** with excellent gonadotropin releasing activity of the formula pGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Glu-NH2 was isolated. Thus, 10,000 chicken hypothalamus were boiled in **acetic acid**, homogenized, and centrifuged at 10,000 r.p.m. for 1 h.

Approx. 40 L of supernatant were subjected to gel chromatog. with Sephadex G-25 after desalting and concentrating by ultrafiltration. Fractions containing gonadotropin releasing activities were eluted and subjected to further fractionation by HPLC with IEX 530 SIL having a 4 + 250 column. Gonadotropin releasing activity was found in fractions have an ionic strength of μ = 0.08 or μ = 0.3. The fraction eluted at μ = 0.3 contained a previously unknown peptide with gonadotropin releasing activity. Approx. 7 μg of the **decapeptide** was **purified** from .apprx.10 kg of chicken hypothalamus.

L60 ANSWER 13 OF 28 MEDLINE on STN DUPLICATE 5
ACCESSION NUMBER: 86033942 MEDLINE
DOCUMENT NUMBER: PubMed ID: 3863818
TITLE: Pertussis toxin-catalyzed ADP-ribosylation of transducin. Cysteine 347 is the ADP-ribose acceptor site.
AUTHOR: West R E Jr; Moss J; Vaughan M; Liu T; Liu T Y
SOURCE: Journal of biological chemistry, (1985 Nov 25) 260 (27) 14428-30.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198512
ENTRY DATE: Entered STN: 19900321
Last Updated on STN: 20021218
Entered Medline: 19851220

AB Pertussis toxin catalyzes the transfer of ADP-ribose from NAD to the guanine nucleotide-binding regulatory proteins Gi, Go, and transducin. Based on a partial amino acid sequence for a tryptic peptide of ADP-ribosylated transducin, asparagine had been characterized as the site of pertussis toxin-catalyzed ADP-ribosylation. Subsequently, cDNA data for the alpha subunit of transducin indicated that the putative asparagine residue was, in fact, not present in the protein. To determine the amino acid that served as the ADP-ribose acceptor, radiolabel from [adenine-U-14C]NAD was incorporated, in the presence of pertussis toxin, into the alpha subunit of transducin (0.3 mol/mol). An ADP-ribosylated, tryptic peptide was **purified** and fully sequenced by automated Edman degradation. The amino acid sequence, Glu-Asn 343-Leu-Lys-Asp 346-X-Gly 348-Leu-Phe, corresponds to the cDNA sequence coding the carboxyl-terminal **nonapeptide**, Glu 342-Phe 350, which includes by cDNA sequence cysteine at position 347. Neither Asn 343 nor Asp 346 appeared to be modified; residue 347 adhered to the sequencing resin. Cysteine, the missing residue, was eluted from the sequencing resin with **acetic acid** along with 76% of the peptide-associated radioactivity, half of which, presumably ADP-ribosylcysteine, eluted from an anion exchange column between NAD and ADP-ribose; the other half had a retention time corresponding to 5'-AMP. We conclude that Cys 347 and not Asn 343 or Asp 346 is the site of pertusis toxin-catalyzed ADP-ribosylation in transducin.

L60 ANSWER 14 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1985:181760 CAPLUS
DOCUMENT NUMBER: 102:181760
TITLE: HPLC of protected peptide segments
AUTHOR(S): Pedroso, Enrique; Grandas, A.; Amor, J. C.; Giralt, E.
CORPORATE SOURCE: Fac. Quim., Univ. Barcelona, Barcelona, Spain
SOURCE: Pept., Proc. Eur. Pept. Symp., 18th (1984), 133-6.
Editor(s): Ragnarsson, Ulf. Almqvist & Wiksell:

Stockholm, Swed.
CODEN: 53PWAN
DOCUMENT TYPE: Conference
LANGUAGE: English
AB The use of reversed-phase HPLC is described for following the progress of stepwise solid-phase synthesis of peptides, for analyzing crude reaction mixts. of synthetic protected peptides, and for the semipreparative **purification** of the protected peptide segments. The method was applied to the fully protected synthetic **nonapeptide** corresponding to the 35-43 segment of toxin II from the scorpion Androctonus australis. Radial-Pak C18 and Radial-Pak CN cartridges were used for the anal. studies and semipreparative sepns. were carried out on an Ultrasphere ODS column. The mobile phase for linear gradient elution contained **propionic acid** and, in addition, DMF for semipreparative sepns. Chromatograms on C18 and CN packings showed dramatic differences in selectivity, efficiency, and resolving power.

L60 ANSWER 15 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1984:204284 CAPLUS
DOCUMENT NUMBER: 100:204284
TITLE: Analogs of the releasing factor of luteinizing and follicle-stimulating hormones, analogs having a high gonadotropic activity
INVENTOR(S): Flegel, Martin; Pospisek, Jan; Picha, Josef; Pichova, Drahomira; Krojidlo, Milan; Kolinsky, Jiri
PATENT ASSIGNEE(S): SPOFA Spojene Podniky pro Zdravotnickou Vyrobu, Czech.
SOURCE: Belg., 18 pp.
CODEN: BEXXAL
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
BE 897455	A1	19831201	BE 1983-211294	19830803
CS 230614	B	19840813	CS 1982-5868	19820806
AT 8302715	A	19880415	AT 1983-2715	19830726
AT 387026	B	19881125		
SE 8304158	A	19840207	SE 1983-4158	19830727
SE 456345	B	19880926		
SE 456345	C	19890126		
FR 2531952	A1	19840224	FR 1983-12707	19830802
FR 2531952	B1	19880722		
GB 2125408	A1	19840307	GB 1983-20922	19830803
GB 2125408	B2	19860618		
DE 3328235	A1	19840405	DE 1983-3328235	19830804
JP 59059654	A2	19840405	JP 1983-141977	19830804
HU 31613	O	19840528	HU 1983-2783	19830805
HU 192962	B	19870828		
CA 1206959	A1	19860701	CA 1983-434011	19830805
CH 658662	A	19861128	CH 1983-4271	19830805
US 4512923	A	19850423	US 1983-521108	19830808

PRIORITY APPLN. INFO.: CS 1982-5868 A 19820806
AB Analogs of LH-RH [9034-40-6] and FSH-RH were prepared, which had a high gonadotropic activity and corresponded to the general structure pGlu-His-Trp-Ser-Tyr-D-Tle-Leu-Arg(X)-Pro-NH-Et, where pGlu = pyrc acid, D-Tle = tert-leucine, and X = H or a protecting group, prefe p-toluenesulfonyl radical. Thus, the protecting group was removed the hexapeptide Z-Ser-Tyr-D-Tle-Leu-Arg(Tos)-Pro-NH-Et [88462-78-

g) by hydrogenolysis on a Pd catalyst in a MeOH solution, to obtain 3.2 g of a substance containing 88% free hexapeptide. A condensation reaction was then effected in an anhydrous mixture of DMF and DMSO (1:1) using 1.6 g pGlu-His-Trp-N2H3 [51952-33-1], 3.2 g of the free hexapeptide, and 0.5 mL n-Bu nitrite, at -20° for 30 min. The pH of the reaction mixture was adjusted to 8-9, and the mixture was refrigerated for 4 days. After evaporation of the solvents, the residue was dissolved in a small volume of ether, and the oily solution thus obtained was diluted with 30 mL MeOH. The product was precipitated by addition of **Et acetate**, recovered by filtration, and washed with an **Et acetate**-ether mixture, to obtain 3.65 g of the crude **nonapeptide** pGlu-His-Trp-Ser-Tyr-D-Tle-Leu-Arg(Tos)-Pro-NH-Et [88462-75-3]. The substance was **purified** by preparative chromatog.; a sample of 300 mg crude **nonapeptide** gave 148 mg of a 96% pure product.

L60 ANSWER 16 OF 28 MEDLINE on STN
ACCESSION NUMBER: 83104956 MEDLINE
DOCUMENT NUMBER: PubMed ID: 6295746
TITLE: Partial isolation and characterization of testicular GnRH-like factors.
AUTHOR: Bhasin S; Heber D; Peterson M; Swerdloff R
SOURCE: Endocrinology, (1983 Mar) 112 (3) 1144-6.
Journal code: 0375040. ISSN: 0013-7227.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 198303
ENTRY DATE: Entered STN: 19900318
Last Updated on STN: 19970203
Entered Medline: 19830324

AB We report here partial isolation and characterization of at least two GnRH-receptor binding factors from the ethanol: chloroform: **acetic acid** (ECA) extracts of rat testis. The displacement curve of defatted, steroid-free and desalted ECA extract was parallel to that of D-(leu)6-des (Gly)10-GnRH-EA in a GnRH-radioreceptor assay. Immunoaffinity chromatography on cyanogen bromide-activated Sepharose 4B beads covalently bound to an antibody raised against d-(lys)6-GnRH resulted in more than a hundredfold increase in receptor binding specific activity. Equivalent amounts of kidney extract after affinity chromatography showed no significant activity. Coincubation of the material **purified** by affinity chromatography with the labeled ligand did not result in significant peptidase degradation of the label, indicating that apparent displacement of the label in the receptor assay was not the result of cleavage of the ligand. HPLC of the material partially **purified** by affinity chromatography on a reverse phase 5 micron ODS column revealed two peaks of receptor binding activity. Preliminary estimates of molecular weights of these factors based on SDS-PAGE and gel filtration are 68,000 and 6,000 respectively. We conclude that there are at least two factors in rat testis with GnRH-receptor-binding properties that are chemically distinct from the native **decapeptide.**

L60 ANSWER 17 OF 28 MEDLINE on STN DUPLICATE 6
ACCESSION NUMBER: 82265778 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7050119
TITLE: Structure of chicken hypothalamic luteinizing hormone-releasing hormone. II. Isolation and characterization.

AUTHOR: King J A; Millar R P
SOURCE: Journal of biological chemistry, (1982 Sep 25) 257 (18) 10729-32.
Journal code: 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198210
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19900317
Entered Medline: 19821029

AB Avian luteinizing hormone-releasing hormone (LH-RH) has been isolated from 249,000 chicken hypothalami and shown to differ structurally from mammalian hypothalamic LH-RH. **Purification** was achieved by **acetic acid** extraction, anti-LH-RH affinity chromatography, and cation exchange and reverse phase high performance liquid chromatography. The isolated peptide eluted as a single peak on reverse phase high performance liquid chromatography. Acid hydrolysis of the peptide yielded integral molar ratios of amino acids and a composition identical with that of mammalian **decapeptide** LH-RH, except for the presence of an additional glutamic acid residue and the absence of arginine. The isoelectric point of chicken LH-RH (7.3) is consistent with the glutamic acid representing a glutamine residue. We therefore synthesized [Gln8]LH-RH and established that it has chromatographic properties identical with natural chicken LH-RH. These studies indicate that the structure of chicken hypothalamic LH-RH is: pGlu-His-Trp-Ser-Tyr-Gly-Leu-Gln-Pro-Gly-NH2.

L60 ANSWER 18 OF 28 MEDLINE on STN DUPLICATE 7
ACCESSION NUMBER: 83006150 MEDLINE
DOCUMENT NUMBER: PubMed ID: 7118412
TITLE: Solid-phase synthesis of peptides via alpha, beta-unsaturated amino acids: oxytocin, simultaneous incorporation of amide functions in COOH-terminal and endo-positions.
AUTHOR: Noda K; Gazis D; Gross E
SOURCE: International journal of peptide and protein research, (1982 Apr) 19 (4) 413-9.
Journal code: 0330420. ISSN: 0367-8377.
PUB. COUNTRY: Denmark
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198212
ENTRY DATE: Entered STN: 19900317
Last Updated on STN: 19900317
Entered Medline: 19821203

AB Oxytocin was synthesized via the solid-phase method using dehydroalanine as pseudo-protecting group of the carboxyl-terminal as well as the omega-amide functions of asparagine and glutamine in endo-position. Starting with Boc-Gly-Dha-resin and using Boc-L-Asp(Dha-NHEt)-OH and Boc-L-Glu(Dha-NHEt)-OH as precursors of asparagine and glutamine, respectively, oxytocin was assembled in stepwise manner under solid phase synthesis conditions. Treatment of the protected [Glu(Dha-NHEt)4, Asp(Dha-NHEt)5]-oxytocin-Dha-resin with 1 n HCl in glacial **acetic acid** in the presence of 3 equivalent water removed the peptide from the support with the simultaneous formation of the asparagine and glutamine residues to give the protected **nonapeptide** amide: Cbz-Cys(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH2, which was

deprotected with sodium in liquid ammonia and then oxidized with diiodoethane to give oxytocin. After **purification** by gel chromatography and countercurrent distribution, the product displayed the chemical and physical properties and oxytocic activity (533 +/- 301U/mg) of a standard oxytocin preparation.

L60 ANSWER 19 OF 28 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN
ACCESSION NUMBER: 1982:171172 BIOSIS
DOCUMENT NUMBER: PREV198273031156; BA73:31156
TITLE: RAT TESTIS IMMUNO REACTIVE LHRH DIFFERS STRUCTURALLY FROM HYPOTHALAMIC LHRH.
AUTHOR(S): DUTLOW C M [Reprint author]; MILLAR R P
CORPORATE SOURCE: DEP CHEM PATHOL, UNIV CAPE TOWN MED SCH, OBSERVATORY 7925, S AFR
SOURCE: Biochemical and Biophysical Research Communications, (1981) Vol. 101, No. 2, pp. 486-494.
CODEN: BBRCA9. ISSN: 0006-291X.
DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH
AB LHRH immunoreactivity (LHRH-IR) was identified in **acetic acid** extracts of adult rat testes and partially **purified** by immunoaffinity chromatography. On Sephadex G-100 this material separated into 4 major peaks of > 100, .apprx. 32, .apprx. 5 and ≤ 4K [kilodaltons]. The ≤ 4K peak of LHRH-IR eluted later than synthetic hypothalamic LHRH **decapeptide** on Sephadex G-25. Antibody binding studies on the various LHRH-IR species with antisera specific for different regions of synthetic LHRH **decapeptide** indicate that all the testicular LHRH-IR molecules have C-terminal immunological homology with the hypothalamic **decapeptide** but differ towards the N-terminus of the **decapeptide** sequence.

L60 ANSWER 20 OF 28 MEDLINE on STN DUPLICATE 8
ACCESSION NUMBER: 79020761 MEDLINE
DOCUMENT NUMBER: PubMed ID: 697727
TITLE: Influence of the peptide-chain length on disulphide-bond formation in neurohypophysial hormones and analogues.
AUTHOR: Moore G
SOURCE: Biochemical journal, (1978 Aug 1) 173 (2) 403-9.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 197811
ENTRY DATE: Entered STN: 19900314
Last Updated on STN: 19900314
Entered Medline: 19781118
AB (8-Arginine)vasopressin, (8-arginine)vasotocin, oxytocin and oxypressin, the 'ring' derivatives pressinamide and tocinamide, and the extended-chain analogues Pro-Arg-Val-(8-arginine)vasopressin and (8-arginine)vasopressinoyl-Ala-Met-Ala-NH(2), were synthesized by the solid-phase method and **purified** by sequential gel filtration on Sephadex G-15 in 50% **acetic acid** and 0.2M-**acetic acid.** Controlled oxidation of the thiol groups of the reduced peptides obtained after deprotection with sodium in liquid ammonia gave rise to products that depended on the length of the peptide chain: (i) **nonapeptides** gave monomer and dimer species, (ii) hexapeptides produced mixtures containing higher polymers, and (iii)

dodecapeptides gave predominantly monomer with some dimerized material. The evidence suggests that the presence of the acyclic tail tripeptide in the **nonapeptide** hormones induces a conformation in the preceding hexapeptide that favours the formation of an intramolecular disulphide bond. For (8-arginine)vasopressin, intramolecular disulphide-bond formation is enhanced by extension of the peptide chain from either the N- or the C-terminus. The possible significance of these studies to neurohypophysial hormone-prohormone relationships is discussed.

L60 ANSWER 21 OF 28 MEDLINE on STN DUPLICATE 9
ACCESSION NUMBER: 76117687 MEDLINE
DOCUMENT NUMBER: PubMed ID: 765120
TITLE: Re-examination of porcine and bovine hypothalamic fractions for additional luteinizing hormone and follicle stimulating hormone-releasing activities.
AUTHOR: Schally A V; Arimura A; Redding T W; Debeljuk L; Carter W; Dupont A; Vilchez-Martinez J A
SOURCE: Endocrinology, (1976 Feb) 98 (2) 380-91.
Journal code: 0375040. ISSN: 0013-7227.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 197604
ENTRY DATE: Entered STN: 19900313
Last Updated on STN: 19900313
Entered Medline: 19760423

AB More than 150 hypothalamic fractions were reassayed for luteinizing hormone-releasing hormone (LHRH) and follicle stimulating hormone-releasing hormone (FSHRH) activities in search for LHRH and FSHRH which differ from the **decapeptide** (pyro)Glu-His-Trp-Ser-Try-Gly-Leu-Arg-Pro-Gly-NH2 (I). Among the porcine fractions tested were those obtained: 1) from the isolation of thyrotropin-releasing hormone; 2) from two isolation procedures for LHRH; and 3) from methanolic and aqueous 2N **acetic acid** extracts which were subjected to Biogel P-2 filtration and partition chromatography. Some bovine hypothalamic fractions were also tested. Both in vivo and in vitro assays were used for measuring LHRH and FSHRH activities. The values obtained were in each case compared with those resulting from the administration of pure natural or synthetic LHRH **decapeptide** I. A radioimmunoassay for LHRH (I) was also utilized for some fractions. In all the **purification** steps the location of LHRH and FSHRH activity, as determined by in vivo assays, corresponded to that of the **decapeptide** I. **Purification** of hypothalamic extracts on Biogel P-2 and by partition chromatography separated a fraction from the **decapeptide** I, which released more FSH than LH in vitro from the pituitaries of immature female rats. However, this material was inactive in vivo and in other in vitro systems, so that its significance is obscure. The results suggest that if material with LHRH and FSHRH activity other than the **decapeptide** I is present in acid extracts of porcine hypothalami, then its FSHRH and LHRH activity would be a minor part of the total LHRH/FSHRH activity in the extracts. (Pyro)-Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH2 appears to account for most of all of the LHRH and FSHRH activity found.

L60 ANSWER 22 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1968:30057 CAPLUS
DOCUMENT NUMBER: 68:30057
TITLE: Syntheses of polypeptides and their derivatives with N-carboxyamino acid anhydride or N-thiocarboxyamino

acid anhydride in water

PATENT ASSIGNEE(S): Merck and Co., Inc.

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US 3435046	A	19690325	US 1965-504978	19651024
US 3467667	A	19690916	US 1966-544358	19660422
US 3459760	A	19690805	US 1966-545857	19660428
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US 1965-469310	A	19650702
US 1965-504978	A	19651024
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AB The title compds. are prepared in a controlled stepwise process in which the intermediate products formed need not to be separated Amino acids, peptides, or their derivs. containing a free amino group are converted with a N-carboxyamino acid anhydride, N-thiocarboxyamino acid anhydride or a derivative thereof in an aqueous medium with careful control of the pH of the solution in the presence of a borate buffer and the product thus obtained converted with another anhydride under the same conditions and this process repeated until the peptide desired is obtained. Products of high purity were obtained in high yields with a min. formation of sideproducts. Thus, a mixture of 420 mg. arginine-HCl in 20 ml. M aqueous KBO_2 buffer containing 5 g. ice and pH 11 was cooled to 0°, 386 mg. solid N-carboxyphenylalanine anhydride added with vigorous stirring, the components mixed 1 min., the solution acidified to pH 3 with concentrated H_2SO_4 at 0°, and N passed through the mixture for 10 min. to yield Phe-Arg. The mixture was then alkalized to pH 9.0 with concentrated KOH while cooled to 0°, 5 g. ice added, 285 mg. N-carboxyproline anhydride added, the

components mixed 3 min., the mixture decarboxylated with separation of the components, and the same process repeated at pH 10 with the addition of 235 mg. N-carboxyalanine-HCl, mixing 1 min., and decarboxylation to yield Ala-Pro-Phe-Arg. This product gave in turn with 235 mg. N-carboxyalanine anhydride at pH 10 Ala-Ala-Pro-Phe-Arg. The latter treated with 294 mg. N-carboxyvaline anhydride at pH 10 gave Val-Ala-Ala-Pro-Phe-Arg (I). The solution was alkalized to pH 7.5 with NaOH, passed into a column containing Amberlite IRC-50 resin in acid form at 1.0 ml./min., the column washed with 200 ml. H2O (2 ml./min.) and eluted with 75 ml. 0.25N H2SO4 and 75 ml. N H2SO4, the eluate collected in fractions of 25 ml. at 1 ml./min. and samples of all fractions chromatographed in 6:3:1 sec-BuOH-H2O-AcOH indicating the presence of a hexapeptide, the fractions combined and concentrated in vacuo, the pH of the solution adjusted to 7.5, the product freeze-dried, the solid extracted 4 times with 10 ml. vols. MeOH, MeOH removed, the residue dissolved in 6 ml. H2O, the solution acidified to pH 3.0 and the product precipitated with 25 ml. MeOH and then with 55 ml. EtOH, and the suspension stored overnight in a refrigerator and filtered to give I. Similarly were prepared the following polypeptides: Asp-Gly-Gly-Leu; Tyr-Leu-Phe; Val-Leu-Ala-Cys; Lys-Gly-Gly-Leu acetate; Gly-Phe-Leu, Leu(N-Me)-Gly-Gly-Gly-Gly-Ile; Leu-Val-Ala-Ala-Pro-Phe-Arg; Leu-Ala-Gly-Pro-Phe-Arg; Pro-Phe-Arg; Gly-Pro-Phe-Arg; Ala-Gly-Pro-Phe-Arg; Leu-Ala-Gly-Pro-Phe-Arg; Leu-Ala-Gly-Pro-Phe-Arg; Gly-Pro-Phe-Arg; Ala-Gly-Pro-Phe-Arg; Leu-Ala-Gly-Pro-Phe-Arg; Gly-Pro-Phe-Arg; Cys-Gly-Gly-Gly; Ala-Ala-Ala-Ala-Gly. Also synthesized was α-corticotropin as found in sheep. In the first step tetrahydropyranyl ether of N-carboxytyrosine anhydride was coupled with serine and the N-carboxy anhydrides of tyrosine, serine, methionine, histidine, and arginine were used in the following steps to form peptide A. The latter was then converted into peptide AB with the anhydrides of tryptophan, glycine, lysine, proline, valine, glycine, lysine, lysine, arginine, and arginine; peptide ABC was obtained with the anhydrides of proline, valine, lysine, valine, tyrosine, proline, alanine, glycine, glutamic acid, aspartic acid, and aspartic acid; and peptide ABCD was prepared by reaction of the anhydrides of glutamic acid, alanine, serine, glutamine, alanine, phenylalanine, proline, leucine, glutamic acid, and phenylalanine, whereafter the carbobenzoxy and benzyl groups were removed from the product by reduction with Na in liquid NH3. Arginine was separated from a peptide with the aid of carboxypeptidase B. A mixture of 2 cc. of a mixture of 100 cc. tris buffer [NaCl concentration 0.1M and aminotris(hydroxymethyl)methane concentration of 0.2M] of pH 7.65, 1 cc. 8.5 mg. Leu-Ala-Gly-Pro-Phe-Arg sulfate in 3 cc. of the same buffer, and 1 cc. of an enzyme mixture (10 cc.) obtained by diluting 0.015 cc. of a carboxypeptidase-B mixture containing 10 mg. enzyme/ml. with the same buffer, was kept 1 hr. at 37° and chromatographed to give arginine and the pentapeptide. Also prepared was Val-Ala-Gly-Pro-Phe-Arg(14C) from a mixture containing 0.392 mg. Arg-14C (0.5 mc.) in 5.0 ml. 0.01N HCl and 421 mg. arginine in 20 ml. of a N K phosphate buffer of pH 16.5. To 0.2 ml. of this mixture was added rapidly 401 mg. (excess of 5 mole %) N-carboxyanhydride of phenylalanine at 0-2°, the mixing continued 1 hr., the pH kept at 10.5-10.4, a drop capryl alc. added as anti-foaming agent, the pH reduced to 3.5 with concentrated H2SO4, N passed through, the pH increased to 10.2, and 0.1 ml. of the mixture containing Pro-Arg(14C) added with vigorous stirring to 300.5 mg. (excess of 6.5 mole %) of the N-carboxyanhydride of proline at 0-2°, and the mixture allowed to react 1 min., decarboxylated, and further treated with the N-carboxyanhydrides of alanine and arginine to

give the radioactive hexapeptide. A solution of 3 millimoles phenylalaninamide in 30 ml. KBO2 buffer at pH 10.2 was cooled to 0°, 3 millimoles N-carboxyaspartic acid anhydride added with stirring, the components allowed to react 1 min., the solution decarboxylated at pH 4, kept 4 min. at pH 4, the solution alkalized to pH 10.2, cooled to 0°, 3.45 millimoles N-carboxymethionine anhydride added, after 3 min. the mixture decarboxylated at pH 4 with N for 15 min., the precipitate alkalized to pH 10.2, cooled to 0°, treated with 4.6 millimoles N-carboxytryptophane anhydride and decarboxylated, and the product chromatographed to give Me-Asp-Phe-NH2. Also described was the preparation of Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg. First prepared was the tetrapeptide Ser-Pro-Phe-Arg from Arg.HCl and N-carboxyphenylalanine anhydride, N-carboxyproline anhydride, and O-trifluoroacetyl-N-carboxyserine anhydride in Me2CO; the tetrapeptide was then treated with N-carboxyphenylalanine anhydride. Pro-Pro-Gly was then prepared The latter (2.5 g.) at pH 10 was cooled to 0-5°, 1.75 g. carbobenzoxychloride added dropwise with vigorous stirring at 0° and pH 10, the mixture stirred 30 min. after reaching constant pH, extracted twice with 10 ml. Et2O, and acidified with 2.5N HCl to yield an oil which was crystallized from CHCl3. Z-Pro-Pro-Gly (Z = PhCH2O2C) (2.85 g.) was dissolved in 15 ml. dioxane, 800 mg. hydroxysuccinimide added, 1.4 g. N,N'-dicyclohexylcarbodiimide added, the mixture stored overnight in a refrigerator, dicyclohexylurea filtered off and washed with dioxane, the filtrate concentrated, and the pentapeptide crystallized from EtOAc-petr. ether. A solution of 652 mg. of the pentapeptide and 168 mg. NaHCO3 in 2 ml. H2O was prepared, 50 mg. of the N-hydroxysuccinimide ester of Z-Pro-Pro-Gly in 3 ml. EtOH added, the mixture kept overnight at room temperature and acidified to pH 2 with HCl, EtOH removed, the precipitated oil separated from the aqueous phase and dissolved in EtOAc, and the solution washed with H2O, dried, and evaporated to dryness to give 565 mg. of the carbobenzoxy-protected octapeptide. The latter (485 mg.) was dissolved in 10 ml. glacial AcOH, 500 mg. 10% Pd-C added, the mixture hydrogenated 2 hrs. at 2.8 kg./cm.2, the product dissolved in 30 ml. glacial AcOH, 500 mg. 10% Pd-C added, and the mixture again hydrogenated at 2.8 kg./cm.2 overnight to give 167 mg. Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg. The latter (48 mg.) was dissolved in 1.8 ml. buffer of pH 10, the solution cooled to 0°, pH changed to 9.5, 0.3 ml. (an excess of 100%) N-carboxyarginine anhydride in HCONMe2 added, the pH changed to 6.3 after mixing for 1 min., the mixture dried, and the solid obtained with freeze-drying desalted by extraction with MeOH and passed through a column with Amberlite IRC-50 resin, and the **nonapeptide** obtained by elution with 25-50% AcOH in H2O. Freezedrying of fractions 13-15 gave 17.9 mg. bradykinin; this was dissolved in 7 ml. MeOH, and the solution centrifuged, concentrated at room temperature, and centrifuged to give an amorphous product which was washed with Et2O and dried. Spinco amino acid analysis indicated the bradykinin structure. A mixture of 2.02 millimoles Phe-Gly-Leu-Val-Gly and 30 ml. KBO2 buffer of pH 10 was cooled to 0°, 3 millimoles N-carboxy-β-(2,4-dihydroxyphenyl)-L-alanine bis(tetrahydropyranyl) ether added, the components mixed and allowed to react 1 min., the pH adjusted to 4 under passage of N through the mixture 20 min., the temperature raised to room temperature, and the mixture kept 1 hr. at room temperature and chromatographed to give L-Ala[β-3,4-(HO)2C6H3]-Phe-Gly-Leu-Val-Gly. Also prepared were Tyr-Leu-D-Phe, Ile-Val-Gly-substituted epinephrine, and Gly-Leu-Val-substituted α-naphthylamine. To a mixture of 2 millimoles leucine in 20 ml. KBO2 buffer of pH 10 was added with vigorous stirring at 25° 2.05 millimoles solid N-thiocarboxyglycine anhydride, the

reaction continued 1 min., the solution acidified with concentrated HCl to pH 5 for dethiocarboxylation, N passed through the mixture 10 min., and the Gly-Leu obtained further treated with N-thiocarboxyglycine anhydride and N-thiocarboxyphenylalanine anhydride and salted out with (NH4)2SO4 to yield Phe-Gly-Gly-Leu. Similarly were prepared Tyr-Leu-Phe, Val-Leu-Ala-Cys, Lys-Gly-Gly-Leu acetate, Gly-Phe-Leu, Gly-Pro-Phe-Arg, bradykinin, Gly-Leu-Phe-Arg(14C), and His-Ala-Leu-Gly-Ile. To 3.93 g. N-carboxyserine anhydride in 200 ml. dioxane was added 5 ml. (F3CCO)2O, the solution stirred 1.75 hrs. at 25° and concentrated, the residue dissolved in dioxane, and the solvent removed to give trifluoroacetyl-N-carboxyserine anhydride. Cl3CCOCl (4 ml.) and 3.93 g. N-carboxyserine anhydride in 200 ml. dioxane gave after stirring 16 hrs. at 25° trichloroacetyl-N-carboxyserine anhydride. A mixture of 84 g. serine, 75 ml. EtOH, 18 ml. H2O, and 68 ml. of a 11.7N KOH solution was mixed under N with 97.5 g. methylethylxanthate at 25-30°, the mixture kept 2 hrs. at 25-30°, heated to 45° 0.5 hr., kept at 45°, alc. removed, 160 ml. H2O added, the mixture extracted twice with 100 ml. Et2O, the alkaline aqueous layer covered with 100 cc. EtOAc, 73 ml. 12N HCl and 70 ml. H2O added, and the mixture marked up to yield methylthionourethaneserine. The latter (8 g.) was dissolved in 40 ml. tetrahydrofuran, 6.1 ml. PBr3 added with stirring at 0-5°, and the mixture worked up to give N-thiocarboxyserine anhydride after removal of the solvent, 5.8 g. of the anhydride dissolved in 200 ml. dioxane, 5 ml. (F3CCO)2O added, and the solution stirred 2 hrs. at 25° and concentrated to give trifluoroacetyl-N-thiocarboxyserine anhydride. Similarly was prepared the trichloroacetyl derivative Trichloromethylsilane (4.5 ml.) was added slowly to 5.1 g. N-carboxythreonine anhydride in 75 ml. anhydrous tetrahydrofuran under N, while the temperature was kept at 2°, 2.98 ml. anhydrous 4-methylthiazole added slowly, the temperature of the solution raised to room temperature, the solution filtered under N, and the filtrate worked up under N to give O-trimethylsilyl-N-carboxythreonine anhydride (II) Similar anhydrides were prepared from serine, tyrosine, and β-phenylserine. A solution containing 145 mg. phenylalanine in 10 ml. H2O and 10 ml. 0.9M H3BO4 was mixed with 50% NaOH until pH 10.5, 326 mg. II added portionwise with stirring over 3 min., the mixture kept at 5° and the pH at 10.5 with 0.75 ml. 2.5N NaOH, the pH of the solution reduced to 3 with H2SO4, the temperature raised to 30°, and the mixture kept 5 min. at 30° for decarboxylation. Paper electrophoresis at pH 9.5 in a 0.1M Na tetraborate solution at 600 v. for 3 hrs. indicated the presence of Thr-Phe. Similarly was prepared Ser-Phe. Also prepared were Thr-Met-Ser(O-PhCH2)-Ile-Thr and Gly-Tyr-Leu-Phe. Stirring a mixture of 1 g. N-carboxytyrosine anhydride in 20 ml. dihydropyran containing 60 mg. p-MeC6H4SO2Cl 48 hrs. at 25° and addition of 30 ml. petr. ether gave tetrahydropyranyl-N-carboxytyrosine anhydride. Variations of the latter method were described. Also described was the preparation of tetrahydropyranyl-N-thiocarboxytyrosine anhydride by first preparing methylthionourethanetyrosine and treating the latter with PBr3 and treating the N-thiocarboxytyrosine anhydride obtained with dihydropyran. Similar reactions could be carried out with 3,5-diiodotyrosine and 3,5-dibromotyrosine. A mixture of 5 g. anhydrous aspartic acid in 200 ml. tetrahydrofuran was treated 20 min. at 25° with COCl2, the mixture kept 10 min., 300 ml. anhydrous EtOAc added, the solution concentrated to 40 ml., 60 ml. EtOAc added, a small amount of precipitate filtered off under N, 85 ml. **hexane** added, the solution stirred 10 min., 120 ml. **hexane** added with stirring, and stirring continued 5 min. precipitated N-carboxyaspartic acid anhydride (III). The following results were

obtained in other prepns. of III (solvent, amount COCl2, ml. solvent, volume in ml., liquid precipitating agent given): dioxane, -, 200 BuOAc, 40, **cyclohexane**; tetrahydrofuran, 50, 300 EtOAc, 100, **cyclohexane**; dioxane, 100, 200 BuOAc, 100, **hexane**; Et2O, 200, 200 EtOAc, 40, pentane; Pr2O, 100, 1000 PrOAc, 100, isooctane. III was identified by converting it into isoasparagine. A mixture of 2.0 g. benzyloxycarbonylasparagine in 20 ml. dioxane and 0.24 ml. PBr3 was stirred 18 hrs., passed over 50 ml. silica gel previously treated with 1:1 CH2Cl2-dioxane followed by dioxane to give N-carboxyasparagine anhydride. Similarly was prepared N-carboxyglutamine anhydride. To a solution of 13.00 g. serine in 500 ml. distilled H2O was added 14.33 g. Ag2O, the mixture stirred at room temperature, the product filtered off, and the filtrate treated with MeOH under stirring and to give serine Ag chelate. Similarly were prepared the Ag chelate of threonine and Cu, Co, Au, Hg, Ni, and Pt chelates of hydroxylated amino acids. The Ag chelate of serine (21.20 g.) was suspended in 250 ml. dioxane under N, 1.0 mole COCl2 added, the mixture stirred 1 hr. at 70°, cooled, and filtered, the filtrate washed with C6H6, and the filtrate and liquid combined and concentrated by freeze-drying to give N-carboxyserine anhydride. To an aqueous solution of 25.0 g. of a 2:3 mixture of KHCO3 and K2CO3 in 1 l. H2O was added 15.52 g. L-histidine, 54.30 g. HgCl2 added with stirring, the temperature of the solution raised to 65°, stirring continued, the mixture cooled to room temperature to give N'-chloromercurihistidine, 29.44 g. of the product stirred 1.5 hrs. with 100 ml. anhydrous Me2CO, 100 ml. Me2CO added, the mixture cooled with ice while excess COCl2 was added during 40 min., the ice removed, 2 ml. Hg added, the precipitate filtered off, and the solvent distilled from the filtrate to give N-chloromercuri-N-carboxyhistidine anhydride (IV) which was **purified** with EtCOMe. IV (1.292 g.) was added to a solution of 90 mg. alanine in 25 ml. of a 0.2M EDTA buffer at pH 10 and at 0°, the solution acidified after 5 min. to pH 2.5, H2S passed through the mixture, the mixture filtered, N passed through the mixture, the mixture filtered, and the filtrate diluted to 50 ml., alkalized to pH 8, and passed through a column containing C, and His-Ala obtained by chromatog. (1:9 NH3-MeOH) over silica gel. Similarly was prepared Lys-Ala-Leu-Gly-Ile.

L60 ANSWER 23 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1968:78614 CAPLUS
DOCUMENT NUMBER: 68:78614
TITLE: Polypeptides. XXXVIII. Elimination of the histidine residue as an essential functional unit for biological activity of β-corticotropin
AUTHOR(S): Hofmann, Klaus; Bohn, Hans; Andreatta, Rudolf H.
CORPORATE SOURCE: School of Med., Univ. of Pittsburgh, Pittsburgh, PA, USA
SOURCE: Journal of the American Chemical Society (1967), 89(26), 7126-7
CODEN: JACSAT; ISSN: 0002-7863
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The synthesis and biol. properties of 5-glutamine-6-β-(3-pyrazolyl)alanine-β-corticotropin1-20 amide (I), i.e. Ser-Tyr-Ser-Met-Gln-Pyr(3)ala-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-NH2 [Pyr(3)ala = β-(3-pyrazoyl)alanine] are examined to determine the effects of amino acid substitution in β-corticotropin1-20 amide, i.e. Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-NH2, on biol. activity. Phe-Arg-Trp-Gly is acetylated with p-nitrophenyl Nα,NPyr-dibenzyloxycarbonyl-β-(3-

pyrazolyl)alaninate, the resulting condensate hydrogenolyzed into Pyr(3)ala-Phe-Arg-Trp-Gly, the pentapeptide coupled with the azide of BOS-Ser-Tyr-Ser-Met-Gln (BOC = tert-BuO2C), the tosylate salt of the protected **decapeptide** coupled with the tosylate salt of Lys(BOC)-Pro-Val-Gly-Lys(BOC)-Lys(BOC)-Arg-Arg-Pro-Val-NH2 using dicyclohexylcarbodiimide as the condensing reagent, and the protected eicosapeptide amide isolated by chromatog. on Sephadex G25 and deblocked by exposure to 90% F3CCO2H, F3CCO2- exexchanged for H3CCO2- on Amberlite IRA-400, and I **purified** by chromatog. on CM-cellulose. Biol. studies indicate that the characteristic acid-base properties of the imidazole portion of histidine are not essential for both the adrenocorticotropic and melanocyte expanding properties of the β-corticotropin mol.

L60 ANSWER 24 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1966:429728 CAPLUS
DOCUMENT NUMBER: 65:29728
ORIGINAL REFERENCE NO.: 65:5527e-h,5528a-g
TITLE: Peptides. LIII. Synthesis of a **decapeptide** derivative with the sequence 21-30 of the B chain of human insulin
AUTHOR(S): Meienhofer, Johannes
CORPORATE SOURCE: Tech. Hochsch., Aachen, Germany
SOURCE: Justus Liebigs Annalen der Chemie (1966), 692, 231-6
CODEN: JLACBF; ISSN: 0075-4617
DOCUMENT TYPE: Journal
LANGUAGE: German
OTHER SOURCE(S): CASREACT 65:29728

AB cf. preceding abstract The C-terminal **decapeptide** with the sequence 21-30 of the B chain of human insulin was prepared as the protected derivative Z-L-(γ-OBt)Glu-L-(Nω-Tos)Arg-Gly-L-Phe-L-Phe-L-Tyr-L-Thr-L-Pro-L-(Nε-Tos)-Lys-L-(O-Bt)Thr-OBt (I) (Z = PhCH2O2C. Tos = tosyl, Bt = tert-Bu, OBt = tert-Bu ester, OSu = N-hydroxysuccinimide ester, ONP = p-nitrophenyl ester, DCCI = dicyclohexylcarbodiimide, solvent A = 75:13.5:11.5 sec-BuOH-HCO2H-H2O, solvent B = 85:15 sec-BuOH-10% aqueous NH3, and DMF = HCONMe2 throughout this abstract; all amino acids except glycine have the L-configuration). Z-Thr was converted with Me2C:CH2 in CH2Cl2 in the presence of concentrated H2SO4 (method of Beyerman and Bontekoe, CA 58, 5779d) into oily Z-(O-Bt)-Thr-OBt, which hydrogenated in MeOH over Pd black gave oily (O-Bt)Thr-OBt (II), b0.05-0.1 80-90°, Rf 0.87 (A), which was converted into the protected dipeptide Nα-Z-(Nε-Tos)-Lys-(O-Bt)Thr-OBt (III) by the following methods. Method A. Nα-Z-(Nε -Tos)-Lys (IIIa) (80.1 g.) in 1 l. absolute tetrahydrofuran treated at -10° with 25.9 cc. Et3N and 24.4 g. ClCO2Bu-iso, followed after 10 min. by 42.7 g. II in 300 cc. DMF, after 15 hrs. at 20° the mixture worked up, the product dissolved in EtOAc, and the solution washed with cold 0.5N H2SO4, M NaHCO3, and H2O, dried, and evaporated in vacuo gave 116.9 g. oily III. Method B. IV (27.4 g.) in 100 cc. MeCN combined with 14.1 g. II in 100 cc. EtOAc and the solution treated at -5° with 13.4 g. DCCI in 100 cc. MeCN, kept 1 hr. at 0° and 15 hrs. at 20°, and evaporated in vacuo gave, after work-up by method A, 40.5 g. oily III. Method C. To 6 g. II in 100 cc. EtOAc was added 15 g. Nα-Z-(Nε-Tos)Lys-ONP and the solution kept 15 hrs. at 20°, washed 10 times with 0.5N aqueous NH3 and once each time with M citric acid, M NaHCO3, and H2O, dried, and evaporated in vacuo to give 16.9 g. oily III. The crude oils obtained by methods A, B, and C were **purified** by countercurrent distribution (CCD) (all CCDs were effected in the system 5:1:8:2 PhMe-CHCl3-MeOH-H2O, unless otherwise stated) (over 250 transfers; K = 0.2) to give 90% chromatographically homogeneous oily III, Rf 1 (A), 0.85-1.0 (B), Rf (by thin layer

chromatography) 0.6-0.7 (A), 0.55-0.75 (B), 0.6 (MeOH), [α]24D 3.6° (c 1, DMF). Hydrogenation of III in MeOH over Pd black gave chromatographically homogeneous (N4-Tos)Lys-(O-Bt)Thr-OBt (IV), Rf 0.93 (A), converted into Z-Pro-(Nε-Tos) Lys-(O-Bt)Thr-OBt (V) by the following methods. Method A. Z-Pro (2.3 g.) treated with 4.7 g. IV like III (method A) gave 6.9 g. crude oily V. Method B. Z-Pro (4.5 g.), 8.6 g. IV, and 3.7 g. DCCI reacted like III (method B) gave 12.5 g. crude oily V. Method C. Z-Pro-OSu (Anderson, et al., CA 61, 4469f) (62.2 g.) treated with 93 g. IV in 100 cc. EtOAc and 120 cc. DMF, the solution kept 15 hrs. at 0° and 4 hrs. at 20°, diluted with 2 1. H2O, and extracted twice with EtOAc, and the combined organic solns. washed with N H2SO4, M NaHCO3, and H2O, dried, and evaporated in vacuo gave 134 g. crude oily V. The crude oily V obtained by methods A, B, and C were **purified** by CCD, giving a large peak with K = 0.16, which was in agreement with the theoretical distribution curve, and a smaller broad peak with K = 0.35-0.50; in the system 4:6:5:5 EtOAc-**hexane**-MeOH-H2O, the main peak had K = 2.57 and the small peak K = 2.85; from the main peaks was isolated 70% (average) V, [α]24D 37.0° (c 1, DMF); the small peak contained a product with a single tert-Bu group, probably Z-Pro-(Nε-Tos)Lys-Thr-OBt. V (15.1 g.) hydrogenated in MeOH over Pd black gave, after filtration and evaporation in vacuo oily decarbobenzoxylated V, which was dissolved in 40 cc. DMF and the solution (A) cooled to 0°. Z-Thr-NHNH2 (7.0 g.) in 40 cc. 2N HCl and 20 cc. H2O treated at 0° with 1.9 g. NaNO2 in 5 cc. H2O; after 5 min. the azide extracted with 100 cc. cold EtOAc, the extract washed with ice cold M NaHCO3 and saturated aqueous NaCl, dried, added to cold solution A, and kept 48 hrs. at 0° and 24 hrs. at 20°, 500 cc. EtOAc added, the organic phase separated, washed with M citric acid, M NaHCO3, and H2O, dried, and evaporated, and the residual oil (17 g.) **purified** by CCD (over 250 transfers; a main peak with K = 0.22 and a very small peak with K = 0.7 were obtained) gave, from the main peak, 15.1 g. oily Z-Thr-Pro-(Nε-Tos)Lys-(O-Bt)Thr-OBt (VI), [α]25D -31.6° (c 1, DMF). To 100 g. N,O-Z2-Tyr and 25.7 g. N-hydroxysuccinimide (Katsoyannis and Suzuki, CA 59, 10230a) in 150 cc. DMF and 150 cc. EtOAc was added 46 g. DCCI in 150 cc. EtOAc at 0°, the solution kept 1 hr. at 0° and 2 hrs. at 20°, filtered, washed with H2O, M NaHCO3, and H2O, dried, and concentrated in vacuo, and the oily residue recrystd. from iso-PrOH to give 96.2 g. N,O-Z2-Tyr-OSu (VII), m. 133-5°, [α]25D -42.6°, (c 1, DMF). VI (5.8 g.) hydrogenated in 200 cc. MeOH over 5 g. Pd black gave oily decarbobenzoxylated VI, Rf 0.82 (A), which in 100 cc. EtOAc treated with 3.82 g. VII in 10 cc. DMF, the solution kept 15 hrs. at 35° and worked up, and the oily product (7.2 g.) **purified** by CCD (over 250 transfers) gave (from the peak with K = 0.09) 6.4 g. oily N,O-Z2-Tyr-Thr-Pro-(Nε-Tos)Lys-(O-Bt)Thr-OBt (VIII), [α]25D -31.0° (c 1, DMF). VIII (21 g.) hydrogenated in 300 cc. MeOH over 5 g. Pd black gave oily bisdecarbobenzoxylated VIII, which in 80 cc. DMF treated with 7.2 g. Z-Phe-OSu, the solution kept 15 hrs. at 40° and worked up, and the oily product **purified** by CCD (over 250 transfers) gave (from fractions 35-80) 16 g. powder, which was dissolved in 100 cc. EtOAc and the solution diluted with 250 cc. Et2O to give 5 g. Z-Phe-Tyr-Thr-Pro-(Nε-Tos)Lys-(O-Bt)Thr-OBt (IX), m. 132-5° (EtOAc), [α]23D -50.8° (c 1, MeOH). IX (3.1 g.) hydrogenated in 150 cc. MeOH over 4 g. Pd black gave oily decarbobenzoxylated IX, Rf 0.65 (A), which in 50 cc. EtOAc treated with 1.11 g. Z-Phe-OSu (Andersen, et al., loc. cir.) in 10 cc. DMF, the solution kept 15 hrs. at 40° and evaporated in vacuo, and the residual oil kept in 200 cc. EtOAc at 40° gave 2.6 g. Z-Phe-Phe-Tyr-Thr-Pro-(Nε-Tos)Lys-(O-Bt)Thr-OBt (X), m. 155-8° (EtOAc), [α]23D -45.8° (c 1, EtOH), its amino acid analysis showing

Phe 2.00, Tyr 0.82, Thr 2.10, Pro 1.05, Lys not determined X (1.7 g.) hydrogenated in 100 cc. MeOH over 2 g. Pd black gave oily decarbobenzoxylated X, Rf 0.7 (A), which in 20 cc. C5H5N treated with 1.06 g. Z-(γ-OBt)Glu-(Nω-Tos)Arg-Gly [Schnabel, Ann. Chemical 674, 218-25(1964)] and 0.35 g. DCCI, the solution kept 72 hrs. at 20° and evaporated in vacuo, and the residue triturated with EtOAc, filtered, washed with EtOAc, and twice each time with M citric acid, 0.5N aqueous NH3, and H2O, dried, extracted with boiling EtOAc, and recrystd. twice from absolute MeOH gave 1.6 g. I, m. 208-9°, [α]23D -24.8° (c 1, AcOH), its amino acid analysis showing Glu 0.93, Arg 1.00, Gly 1.00, Phe 1.96, Tyr 0.84, Thr 1.94, Pro 0.89, Lys 0.91.

L60 ANSWER 25 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1966:490933 CAPLUS
DOCUMENT NUMBER: 65:90933
ORIGINAL REFERENCE NO.: 65:17044c-h,17045a-f
TITLE: Peptides. LIX. Synthesis of **decapeptide** derivatives of the insulin B-chain, B21-30
AUTHOR(S): Mathur, Krishna Behari; Klostermeyer, Henning; Zahn, Helmut
CORPORATE SOURCE: Tech. Hochsch., Aachen, Germany
SOURCE: Hoppe-Seyler's Zeitschrift fuer Physiologische Chemie (1966), 346(1), 60-8
CODEN: HSZPAZ; ISSN: 0018-4888
DOCUMENT TYPE: Journal
LANGUAGE: German
AB cf. Ann. Chemical 696, 220-5(1966); Z. Naturforsch. b 21(8), 763-73(1966); CA 65, 5527e. (All amino acids have L-configuration throughout this abstract, Z = PhCH2O2C, BOC = tert-BuO2C, TOS = p-MeC6H4SO2, OSu = N-hydroxysuccinimide ester.) A solution of 19 g. Z-Lys-(BOC) in 50 cc. AcOEt was treated with 7.3 g. Ala-OBu-tert and 10.3 g. dicyclohexylcarbodiimide at 0° for 2 days. After separation from dicyclohexylurea, the product was washed with 0.1M citric acid, N NaHCO3, and H2O, dried, and concentrated in vacuo, and the residue crystallized from Et2O, and recrystd. from Me2CO-petroleum ether to give 20 g. Z-Lys-(BOC)-Ala-OBu-tert (I), m. 88-90°, [α]24D -13° (c 1, Me2CO). Using the azide synthesis, I, m. 91-3°, was prepared in 51% yield, false -10°. I (17 g.) was hydrogenated over Pd black in 50 cc. MeOH until no more CO2 was evolved, the product in 50 cc. dioxane treated with 11 g. Z-Pro-OSu at room temperature, the mixture kept overnight, diluted with H2O, and extracted with AcOEt, and the extract washed as described above and concentrated to give 18.4 g. Z-Pro-Lys(BOC)-Ala-OBu-tert (II), m. 132° (AcOEt-petroleum ether), [α]24D - 43° (c 1, HCONMe2). Via the nitrophenyl ester synthesis, the yield was 78%, m. 132-4°, [α]24D -41°. A solution of 15.4 g. Z-Thr(Bu-tert) (**purified** via the dicyclohexylammonium salt) in 50 cc. AcOEt was treated with 6.2 g. N-hydroxysuccinimide in 50 cc. MeCN, and after cooling to 0°, with 10.3 g. dicyclohexylcarbodiimide. The mixture was kept 24 hrs. and worked up to give a sirupy residue. Trituration with Et2O gave 18.3 g.Z-Thr (Bu-tert)-OSu (III), m. 90° (CH2Cl2-petroleum ether), [α]D22 -6.3° (c 1, dioxane). II (15.7 g. was catalytically hydrogenated in 50 cc. MeOH, the product in 50 cc. dioxane treated with 9.8 g. III, and the mixture kept 4 days and worked up from an AcOEt extract to give 16.6 g. Z-Thr(Bu-tert)-Pro-Lys(BOC)-Ala-OBu-tert (IV), m. 75-6° (Et2O-petroleum ether), [α]24D -56.6° (c 1, MeOH). ZTyr(Bu-tert)-OSu (V) was prepared similarly to III from Z-Tyr(Bu-tert) in 85% yield, m. 118°, [α]24D -16.2 (c 1, dioxane). IV (15.2

g.) was hydrogenated in 50 cc. MeOH and the product treated with 8.5 g. V in 50 cc. dioxane and worked up after 4 days as described for IV to give 15.8 g. Z-Tyr(Bu-tert)-Thr- (Bu-tert)-Pro-Lys(BOC)-Ala-OBu-tert (VI), m. 94°, [α]24D -43.1° (c 1, MeOH). Similarly prepared were Z-Phe-Tyr(Bu-tert)-Thr(Bu-tert)-Pro-Lys(BOC)-Ala-OBu-tert (VII) by hydrogenation of VI and condensation of the product with Z-Phe-OSu; yield 98%, m. 110-12°, [α]22D -46° (MeOH), -38.8° (c 1, HCONMe2); Z-Phe-Tyr(Bu-tert)-OMe (VIII) by hydrogenation of Z-Tyr(Bu-tert)-OMe and condensation of the product with Z-Phe-OSu, yield 83%, m. 103-5°, [α]20D -14.7° (c 1, MeOH); and Z-Phe-Phe-Tyr(Bu-tert)-OMe (IX) by hydrogenation of VIII and condensation of the product with Z-Phe-OSu, yield 90%, m. 158-60°, [α]20D -24°. A solution of 20 g. IX in 500 cc. warm MeOH was treated with 14.4 cc. N2H4 hydrate. The crystalline hydrazide, Z-Phe-Phe-Tyr(Bu-tert)-NHNH2 (X), was filtered off the next day, washed with hot MeOH, triturated with Et2O, and finally extracted with hot MeOH to yield 17.2 g., m. 219-21°, [α]22D -10.8° (c 0.5, HCONMe2). Hydrogenation of VII and condensation of the product with Z-Phe-OSu gave 90.2% Z-Phe-Phe-Tyr(Bu-tert)-Thr(Bu-tert)-Pro-Lys(BOC)-AlaOBu-tert (XI), m. 117-18°, [α]22D -46° (c 1, MeOH). To a solution of 2 g. X in 200 cc. HCONMe2 was added at -40°, 1.82 cc. 2.92N HCl-tetrahydrofuran and 0.258 cc. isoamyl nitrite. After 50 min. at -40°, the mixture was neutralized with 0.740 cc. NEt3 and to the cooled solution was added the hydrogenation product of 1.37 g. IV in 25 cc. HCONMe2. After 2 days the product was poured into dilute citric acid solution, extracted with benzene, and crystallized from benzene-**heptane** to yield 1.92 g. XI, m. 11922° (Et2O-petroleum ether), [α]20D -46°. Z-Arg(NO2)-Gly-OBu-tert (XII), prepared from Z-Arg(NO2) and Gly-OBu-tert (dicyclohexylcarbodiimide method) in 58% yield, m. 107-8°, [α]22D -11.1°. XII (2.3 g.) was kept 15 min. in 8 cc. 4N HBr-AcOH and then warmed to complete solution After 1 hr., the hydrobromide was precipitated with absolute Et2O, dried, converted to base in 20 cc. HCONMe2 with NEt3, and kept 2 days with 2 g. Z-Glu(OBu-tert)-OSu. The mixture was diluted and brought to pH 3 with citric acid, the precipitate filtered off, and the product repptd. to give 1.6 g. Z-Glu(OBu-tert)-Arg(NO2)-Gly (XIII), m. 125-7° (aqueous EtOH), [α]22D -10.9° (c 1, HCONMe2). XI (2.6 g.) was hydrogenated and the amorphous product taken up in 30 cc. HCONMe2 and treated with 1.2 g. XIII and 0.41 g. dicyclohexylcarbodiimide. After 4 days at 0° the mixture was filtered, the solution brought to pH 8, the precipitate filtered off, washed with NaHCO3 solution, H2O, and Me2CO, and repptd. from HCONMe2 solution with H2O. The crude product (1.4 g.) was **purified** by countercurrent distribution in the system, toluene-CHCl3MeOH-H2O through 250 steps. The sym. peak with k 0.135 was the **decapeptide** derivative Fractions 18-45 were combined, and 0.7 g. amorphous Z-Glu(OBu-tert)-Arg(NO2)-Gly-Phe-Phe-Tyr(Bu-tert)- Thr (Bu-tert)- Pro- Lys(BOC) - Ala - OBu -tert (XIV) was obtained from MeOH-H2O, m. 203-5°, [α]20D -19.8° (c 0.5, Me2SO). To a solution of 554 mg. vacuum-dried Z-Glu(OBu-tert)-Arg(TOS)-Gly in 10 cc. absolute tetrahydrofuran was added 0.109 cc. NEt3, the mixture cooled to -15°, treated with 0.102 cc. iso-Bu O2CCl, and kept 15 min., a solution of the hydrogenation product of 1 g. XI in 20 cc. tetrahydrofurn added, the mixture kept 30 min. at -150 and at room temperature 1 day, the concentrated solution taken up in AcOEt, the liquid washed with 0.1 M citric acid, N NaHCO3, and H2O, concentrated, and **purified** as for XIV through 324 steps to give a main peak with k 0.085. Fractions 12-33 on working up gave 930 mg. Z-Glu(OBu-tert)Arg(TOS)-Gly-Phe-Phe-Tyr(Bu-tert)-Thr(Bu-

tert)-Pro-Lys(BOC)-Ala-OBu-tert (XV), m. 182-6°, [α]20D -16.4°. Condensation of 5.2 g. Pro-Lys(TOS)-Ala-OBu-tert and 4.0 g. III in 15 cc. MeCN 2 days gave 6 g. oily Z-Thr(Bu-tert)-Pro-Lys(TOS)Ala-OBu-tert, [α]22D -26.5 (c 1, HCONMe2), which was hydrogenated. The product (4.42 g.) in 15 cc. AcOH was kept with 3 g. V 3 days to give 6.4 g. crude Z-Tyr(Bu-tert)-Thr(Bu-tert)-Pro-Lys(TOS)-Ala-OBu-tert (XVI) which crystallized slowly from Et2O-AcOEt-petroleum ether, m. 102-6°, [α]20D -18.8°. Z-Phe- Tyr (Bu-tert)-Thr (Bu-tert)-Pro-Lys (TOS)-Ala-OBu-tert (6 g.) from the condensation of 4.35 g. XVI hydrogenation product and 2.2 g. Z-Phe-OSu, was hydrogenated and the product condensed with 2.1 g. Z-Phe-OSu in 10 cc. HCONMe2 2 days to give 5.3 g. Z-Phe-Phe- Tyr(Bu-tert)- Thr(Bu-tert)- Pro- Lys(TOS)- Ala- OBu-tert (XVII), m. 133-5° (Et2O-petroleum ether), [α]22D- 32.6°. Hydrogenated XVII (2.2 g.), condensed with 1.4 g. Z-Glu(OBu-tert)-Arg(TOS)-Gly in 15 cc. HCONMe2 as in the preparation of XIV gave 1.7 g. Z-Glu(OBu-tert)-Arg(TOS)-Gly-Phe-Phe-Tyr(Bu-tert)-Thr(Bu-tert)-Pro-Lys(TOS)-Ala-OBu-tert (XVIII), m. 182-4°, [α]20D- 19.5°. Hydrogenation of VIII in MeOH over Pd black gave cyclo-Phe-Tyr(Bu-tert) (XIX), m. 269-71°. XIX (1.6 g.) was kept until the next day in CF3CO2H, the mixture concentrated, and the product precipitated from hot pyridine to give 1.2 g. cyclo-Phe-Tyr, m. 283-5° (Me2SO), [α]20D-167° (c 1, HCO2H). IX (5.9 g.) in 45 cc. dioxane and 5 cc. H2O treated 6 hrs. with 8.7 cc. N NaOH gave 2.95 g. Z-Phe-Phe-Tyr(Bu-tert), m. 179- 82°, [α]20D-17° (c 1, MeOH).

L60 ANSWER 26 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1965:403553 CAPLUS
DOCUMENT NUMBER: 63:3553
ORIGINAL REFERENCE NO.: 63:675b-h
TITLE: Polypeptides
PATENT ASSIGNEE(S): CIBA Ltd.
SOURCE: 28 pp.
DOCUMENT TYPE: Patent
LANGUAGE: Unavailable
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
NL 6405541		19641123	NL	
PRIORITY APPLN. INFO.:			CH	19640520

AB Octa-**decapeptides**, their derivs., and salts were prepared by condensation of the hexapeptide benzyloxycarbonyl-γ-tert-butyl-L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophanyl-glycine (I) with the pentapeptide L-seryl-L-prolyl-L-prolyl-tert-butyloxycarbonyl-L-lysyl-L-asparaginic acid di-tert-butyl ester (II) to obtain the protected undecapeptide ester by splitting off the benzyloxycarbonyl group of the latter, and by condensation of the γ-tert-butyl-L-glutamyl-L-histidyl-L-phenylalanyl-L-arginyl-L-tryptophanylglycyl-L-seryl-L-prolyl-L-prolyl-tert-butyloxycarbonyl-L-lysyl-L-asparaginic acid di-tert-butyl ester (III) obtained with tert-butyloxycarbonyl-β-tert-butyl-L-asparagyl-L-serylglycyl-L-prolyl-L-tyrosyl-tert-butyloxycarbonyl-L-lysyl-L-methionine or its azide to yield the protected octadecapeptide ester, and by splitting off the protecting groups with trifluoroacetic acid. As starting materials are also used compds. which contain a glutamine and (or) an asparagine residue instead of a β-glutamyl ester residue and (or) a γ-asparagyl ester residue. The condensation between I and II can be carried out in the presence of a carbodiimide or toluenesulfonic

acid. Thus, 1.271 g. I and 0.237 g. p-toluenesulfonic acid monohydrate were dissolved with heating to 50° in 3.5 cc. dimethylformamide, cooled to 20°, a solution of 0.89 g. II in 3.5 cc. chloroform added, 0.315 g. dicyclohexylcarbodiimide added under stirring, the mixture stirred overnight at 22° and subsequently 8 hrs. at 40°, cooled to 0°, the formed dicyclohexylurea filtered off, the filtrate concentrated at 40°, and the reaction product precipitated as a viscous mass by addition of 35 cc. benzene and 35 cc. petr. ether. The reaction product was dissolved again in 4 cc. methanol and separated as a fine powder by addition of 40 cc. ether, and the precipitate filtered off under strongly reduced pressure and dried at 40°. The crude product thus obtained (2.26 g. tosylate) was dissolved in 32 cc. ethanol and 16 cc. H2O and filtered through a column of a weak basic ion-exchanger (Merck II); the eluate was concentrated to a volume of 50 cc. and subsequently lyophilized. The acetate (2.08 g.) obtained was **purified** to give 1.22 g. of the protected undecapeptide acetate (IV), containing 5% impurities. To prepare III acetate, 1.21 g. IV in 30 cc. methanol was hydrogenated in the presence of 0.04 cc. glacial **acetic acid** and 300 mg. Pd-C (10% Pd) at 30° under atmospheric pressure, without adsorption of the CO2. After 4 hrs. cleaving of the benzyloxycarbonyl group was completed. After removal of the catalyst the product was filtered off, evaporated to dryness, and the amorphous product powdered and dried under strongly reduced pressure at 40° to give 1.076 g. III acetate. Nα-tert-Butyloxycarbonyl-β-tert-butyl-L-asparagyl-L-serylglycyl-L-prolyl-L-tyrosyl-tert-butyloxycarbonyl-L-lysyl-L-methionine hydrazide (814 mg.) was dissolved in 8 cc. absolute dimethylformamide and the container placed into an ice-salt mixture (-12°), where 3.05 cc. N HCl and 0.632 cc. 10% solution of sodium nitrite was added, the solution kept 5 min. at -12° and the azide formed precipitated by addition of 38 cc. 20% NaCl solution precooled to -12°. The precipitate was filtered off at 0° and washed with 10 cc. 5% NaHCO3 solution, subsequently washed with 4 cc. H2O, dissolved in 16 cc. dimethylformamide at 0° and mixed with a solution of 1.07 g. III acetate in 10 cc. dimethylformamide. The solution was kept 18 hrs. at 0°, HN3 removed, the solution kept 23 hrs. at 20°, dimethylformamide evaporated under strongly reduced pressure at 40°, the residue dissolved in 60 cc. hot benzene, precipitated by addition of 60 cc. petroleum ether, filtered off, dried, and **purified** to give 952 mg. protected octadecapeptide acetate, m. 220-5° (decomposition). The products are used as intermediates in synthesis of peptides with longer amino acid chains, as hormones, and as components in pharmaceutical preparations.

L60 ANSWER 27 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1965:22801 CAPLUS
DOCUMENT NUMBER: 62:22801
ORIGINAL REFERENCE NO.: 62:4108a-h,4109a-b
TITLE: Synthesis of eledoisin analogs and homologs. II.L-Seryl-L-lysyl-L-aspartyl-L-alanyl-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methioninamide and L-prolyl-L-seryl-L-lysyl-L-aspartyl-L-alanyl-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methioninamide
AUTHOR(S): Chillemi, Francesco; Goffredo, Onofrio
CORPORATE SOURCE: Farm. Italia S.p.A., Milan
SOURCE: Gazzetta Chimica Italiana (1964), 94(8-9), 866-74
CODEN: GCITA9; ISSN: 0016-5603
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable
OTHER SOURCE(S): CASREACT 62:22801
AB To 15 g. N2-benzyloxycarbonyl-N6-tert-butoxycarbonyl-L-lysine in 200 mL.

Et2O, 9.2 mL. dicyclohexylamine was added and the mixture kept 4 h. at 0° to give 20 g. N2-benzyloxycarbonyl-N6-tert-butoxycarbonyl-L-lysine dicyclohexylammonium salt (XVII), m. 156-9° (EtOH-petr. ether), [α]26D 5° (c 2, EtOH). To 16.8 g. XVII and 7.2 g. XII suspended in 150 mL. CH2Cl2 and cooled to 0° was added 6.6 g. I, the mixture kept overnight and filtered, and the filtrate washed with N HCl, N NaHCO3, and H2O, dried, and evaporated to give 12.5 g. N2-cbo-N6-boc-L-Lys-L-Asp(β-OBu-tert)-OMe (XVIII), m. 78-80° (AcOEt-petr. ether), [α]21D -9 ± 1° (c 2, EtOH). XVIII (5.6 g.) in EtOH solution hydrogenated at 1 atmospheric over Pd-C gave 4.3 g. N6-boc-L-Lys-L-Asp(β-OBu-tert)-OMe (XIX) (sirup), Rf 0.88. To an AcOEt solution of N-tert-butoxycarbonyl-L-serine azide (prepared from 2.2 g. N-tert-butoxycarbonyl-L-serine hydrazide), 4.3 g. XIX in 10 mL. THF was added, the mixture allowed to react 48 h. at 5°, the solvent evaporated, the residue dissolved in AcOEt, and the solution washed with N HCl, N NaHCO3, and H2O, dried (Na2SO4), and evaporated to give 3.5 g. N-boc-L-Ser-L-Lys(N6-boc)-L-Asp(β-OBu-tert)-OMe (XX), m. 80-3° (AcOEt-petr. ether), [α]22D -56 ± 0.5° (c 2, EtOH). XX (2.8 g.) in 10 mL. EtOH was treated with 5.4 mL. N NaOH, the mixture kept 15 min., 30 mL. H2O and 5.5 mL. N HCl were added at 0°, the mixture was extracted with AcOEt, the extract dried (Na2SO4), and the solvent evaporated to give 2 g. N-boc-L-Ser-L-Lys(N6-boc)-L-Asp(β-OBu-tert) (XXI), m. 98-100° (AcOEt-petr. ether), [α]26D -53 ± 1° (c 1, EtOH). To 1.7 g. XXI and 1.8 g. XVI in 20 mL. DMF was added 0.7 g. I at 0°, the mixture kept 5 h. at this temperature and 24 h. at room temperature, and filtered, the solvent evaporated in vacuo, and the residue washed with Et2O to give 2.5 g. crude N-boc-L-Ser-L-Lys(N6-boc)-L-Asp(β-OBu-tert)-L-Ala-L-Phe-L-Ile-Gly-L-Leu-L-Met-NH2, which was converted into the **nonapeptide** L-Ser-L-Lys-L-Asp-L-Ala-L-Phe-L-Ile-Gly-L-Leu-L-Met-NH2 bis(trifluoroacetate) (XXII) by treatment with CF3CO2H. XXII m. .apprx.245° (decomposition), [α]25D -15° (c 2, DMF), Rf 0.68. To 3.9 g. N-benzyloxycarbonyl-L-serine and 7 g. XIX in 150 mL. CH2Cl2 at 0° was added 3.8 g. I, the mixture kept overnight at room temperature, filtered, washed at 0° with N HCl, M NaHCO3, and H2O, and dried, and the solvent evaporated to give 7.9 g. N-cbo-L-Ser-L-Lys(N6-boc)-L-Asp(β-OBu-tert)-OMe (XXIII), m. 102-4°, [α]24D -18° (c 2, EtOH). Hydrogenating 6.5 g. XXIII as described above gave 5.8 g. L-Ser-L-Lys(N6-boc)-L-Asp(β-OBu-tert)-OMe (XXIV) (sirup), Rf 0.82. To 2.1 g. N-tert-butoxycarbonyl-L-proline and 5.2 g. XXIV in 70 mL. CH2Cl2 at 0° was added 2.1 g. I, the mixture kept overnight at room temperature, filtered, washed at 0° with N HCl, M NaHCO3, and H2O, and dried, and the solvent evaporated to give 4.6 g. N-boc-L-Pro-L-Ser-L-Lys(N6-boc)-L-Asp(β-OBu-tert)-OMe (XXV), m. 103-7° (AcOEt-petr. ether), [α]22D -42° (c 2, EtOH). To 2.5 g. XXV in 10 mL. EtOH was added 4.2 mL. N NaOH, the solution kept 15 min. at room temperature, diluted with 25 mL. H2O, cooled to 0°, 4.4 mL. N HCl added, the mixture extracted with AcOEt, and the extract dried and evaporated to give 2.1 g. N-boc-L-Pro-L-Ser-L-Lys(N6-boc)-L-Asp(β-OBu-tert) (XXVI), m. 110°, [α]21D -36° (c 1.4, EtOH). To 2 g. XXVI and 1.8 g. XVI in 35 mL. DMF at 0° was added 0.7 g. I, the solution kept 5 h. at 0° and 24 h. at room temperature, centrifuged, and evaporated, and the residue **purified** by counter-current extraction in 8:3:3:7 MeOH-H2O-CCl4-CHCl3 to give 1.6 g. N-boc-L-Pro-L-Ser-L-Lys(N6-boc)-L-Asp(β-OBu-tert)-L-Ala-L-Phe-L-Ile-Gly-L-Leu-L-Met-NH2, which by hydrolysis with CF3CO2H gave the corresponding **decapeptide**, m. .apprx.250° (decomposition), [α]21D -18° (c 2, DMF), Rf 0.6.

L60 ANSWER 28 OF 28 CAPLUS COPYRIGHT 2005 ACS on STN
ACCESSION NUMBER: 1957:81084 CAPLUS
DOCUMENT NUMBER: 51:81084
ORIGINAL REFERENCE NO.: 51:14554c-i,14555a-i,14556a-b
TITLE: Synthesis of gramicidin S
AUTHOR(S): Schwyzer, R.; Sieber, P.
CORPORATE SOURCE: C I B A A.-G., Basel, Switz.
SOURCE: Helvetica Chimica Acta (1957), 40, 624-39
CODEN: HCACAV; ISSN: 0018-019X
DOCUMENT TYPE: Journal
LANGUAGE: Unavailable

AB The constitution of gramicidin S (I) as a cyclic **decapeptide** (cf. Battersby and Craig, C.A. 45, 7615a) was established (cf. following abstract) by synthesis which is described in detail. The pentapeptide H-Val-p-Tos-Orn-Leu-Phe-Pro-OMe-HCl (cf. Erlanger, et al., C.A. 49, 3012g), for synthesis and abbreviations) was synthesized as an intermediate using activated esters for the preparation of di- and tripeptide derivs. (cf. S., et al., C.A. 51, 1038d). Et3N (10.7 ml.) and 9.0 g. PhCH2CH(NH2)CO2Et.HCl in 50 ml. C4H8O (tetrahydrofuran) treated 5 hrs. at 20° with 15.0 g. L-Me2CHCH2CH(NHOCOCH2Ph)CO2C6H4NO2-p, the solution poured into 400 ml. iced H2O, the product washed with H2O, ice-cold NH4OH [1:1 H2O-NH4OH (d. 0.90)] and H2O, dried in vacuo at 50° and crystallized from C6H6-petr. ether gave 14.4 g. carbobenzoxy-L-leucyl-D-phenylalanine Et ester (II), m. 106°. ClCH2CN (23 ml.), 11.5 ml. NEt3, and 11.44 g. L-PhCH2CH(NHOCOCH2Ph)CO2H mixed with cooling and kept 45 hrs. at room temperature, the solution poured into 500 ml. 2N HCl, the product triturated and filtered off, taken up in Et2O containing a little AcOEt, the washed and dried extract evaporated and the residue crystallized twice from C5H5-petr. ether gave 11.79 g. carbobenzoxy-L-leucyl-D-phenylalanine cyanomethyl ester (III), m. 102-3°. III (11.18 g.), 4.2 g. L-proline Me ester-HCl, 6.5 ml. C4H8O, 8 ml. NEt3, and 0.2 ml. AcOH kept 67 hrs. at room temperature, saponified 45 min. with 1 ml. H2O at room temperature, diluted with much H2O, the oily product taken up in Et2O, the extract washed with dilute NaOH and H2O, evaporated and the residue dried in high vacuum gave 11.43 g. material, taken up in 25 vols. C6H6 and filtered through 10 parts Al2O3, the column washed with C6H6 and the filtrate evaporated yielded 8.7 g. colorless glassy substance. The resin (6.54 g.) in 65 ml. MeOH with addition of 12.5 ml. N HCl hydrogenated 8 hrs. in the presence of 650 mg. 10% Pd-C, filtered, the filtrate evaporated and the residue crystallized from EtOH-Et2O gave 3.8 g. authentic L-leucyl-D-phenylalanyl-L-proline Me ester-HCl (IV), m. 240° (decomposition), [α]D23 -38 ± 4° (c 0.720, MeOH) (cf. Harris and Work, C.A. 45, 7988h). Me2CHCH(NHOCOCH2Ph)CO2C6H4NO2-p (1.32 g.), 1.20 g. L-p-MeC6H4SO2NH (CH2)8CH(NH2)CO2Me, 1.3 ml. NEt3, and 10 drops AcOH in 5 ml. C4H3O stirred 15 hrs. at room temperature, the mixture diluted with EtOAc and washed with H2O, dilute K2CO3 solution, 2N HCl and H2O, the dried organic solution evaporated in vacuo and the residue crystallized from Me2CO-Et2O gave carbobenzoxy-L-valyl-Ndelta;-tosyl-L-ornithine Me ester (V), m. 144-5°; hydrazide (Va), m. 212°. Va converted to the acid azide and treated with IV gave carbobenzoxy-L-valyl-Ndelta;-tosyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-proline Me ester, catalytically reduced to the known L-valyl-Ndelta;-tosyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-proline Me ester-HCl (VI), [α]D24 -48 ± 4° (c 0.9, 0.01N HCl) (cf. E., et al., loc. cit.). VI (100 mg.) in 1.5 ml. CHCl3 kept 5 hrs. at room temperature with 100 mg. Ph3CCl and 5 drops NEt3, evaporated and the residue triturated with 1:1 Et2O-petr. ether, the product taken up in EtOAc, filtered and the crystalline NEt3.HCl washed with cold

tartaric acid and H2O, the dried organic phase evaporated in vacuo, and the residue crystallized twice from C6H6-petr. ether gave 124 mg. trityl-L-valyl-Nδ-tosyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-proline Me ester (VIa), m. 123.5-5.5°. VI (0.615 g.) in 23 ml. dioxane treated with 4.5 ml. N NaOH and 4.5 ml. H2O, diluted with 1.5 ml. MeOH, kept 1 hr. at 36-8° and evaporated in vacuo, the concentrate poured into H2O containing 0.7 g. citric acid, extracted with EtOAc, and the washed and dried extract evaporated in high vacuum yielded 0.503 g. trityl-L-valyl-Ndelta;-tosyl-L-ornithyl-L-leucyl-D-phenylalanyl-L-proline (VII). VI (500 mg.) in 5 ml. EtOAc and 0.5 ml. NEt3 kept 3 min. and filtered, the crystalline NEt3.HCl washed with EtOAc, the filtrate and washings evaporated in vacuo, the residue taken up in 5 ml. AcOEt, the solution treated with 580 mg. VII and 155 mg. 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide (cf. Sheehan and Hlavka, C.A. 51, 4277c) and kept 7 hrs. at room temperature, the mixture diluted with EtOAc washed with N HCl, dilute NH4OH and cold H2O, the dried organic phase evaporated in vacuo, the residue dried in high vacuum and crystallized twice from C6H6-Et2O gave 835 mg. trityl-(Val-p-Tos-Orn-Leu-Phe-Pro)2OMe (L-L-L-D-L)2 (VIII), m. 140-2°, [α]D23 -67 ± 3° (c 0.7, MeOH). VIII (800 mg.) warmed 2 hrs. at 37° with 11 ml. 0.5N NaOH and 3 ml. MeOH, the mixture poured into 900 ml. iced H2O and the solution acidified with citric acid, kept 2.5 hrs. at 0° and filtered, the product washed with H2O and dried gave 600 mg. colorless powder (VIIIa). VIIIa (100 mg.) in 1 ml. AcOH containing 5 drops CF3CO2H and 5 drops H2O kept 10 min. at room temperature and filtered, the filtrate and washings evaporated and the residue chromatographed in 65% alc. over Amberlite IR-4B (previously washed with NH4OH and 65% alc.), the eluate evaporated and the product (55 mg.) slowly crystallized from 60% alc. gave H-(Val-p-Tos-Orn-Leu-Phe-Pro)2-OH (L-L-L-D-L)2 (IX), m. 184-7° (decomposition). Total hydrolysis of IX by boiling 20 hrs. at 110° with 1:1 AcOH-concentrated HCl gave a mixture of ornithine, valine, proline, leucine, and phenylalanine in approx. equal amts. as shown by paper chromatography in PhCH2OH-H2O and detection with ninhydrin (Rf 0.0, 0.28, 0.305, 0.395, 0.421). IX (6 mg.) in 0.5 ml. 85% alc. treated 30 min. at room temperature with 5 mg. 2,4-(O2N)2C6H3F and 1 drop of NEt3, the mixture evaporated and the residue washed repeatedly with 1:1 Et2O-petr. ether, taken up in EtOAc and adsorbed on 0.5 g. neutral Al2O3 (containing 6% H2O), the column washed with EtOAc and eluted with 10:1 MeOH-pyridine, the fraction taken up in the upper phase of 9:1:10 C6H6-PhCH2OH-H2O and rechromatographed on cellulose powder, the rapidly-moving band eluted, the eluate evaporated in high vacuum, the product taken up in 1 ml. alc. and crystallized by diluting the solution with H2O at 70° and cooling slowly to give IX dinitrophenyl derivative, m. 229-30° (decomposition), totally hydrolyzed to yield, by paper chromatography, valine dinitrophenyl derivative (IXa) (Rf 0.935 in 4:1:5 BuOH-AcOH-H2O) in addition to ornithine, valine, proline, leucine, and phenylalanine. IX contains therefore 2 valine groups of which 1 is not in the end position. For comparison, VI was converted into the dinitrophenyl derivative and totally hydrolyzed to yield IXa, ornithine, proline, leucine, and phenylalanine but no free valine. VIIIa (540 mg.) and 500 mg. (p-O2NC6H4)2SO3 in 5 ml. pyridine kept 5 hrs. at room temperature and evaporated, the residue taken up in EtOAc, the solution washed with dilute tartaric acid and H2O, the dried solution evaporated and the residue washed with 1:1 Et2O-petr. ether gave 500 mg. trityl-(Val-p-Tos-Orn-Leu-Phe-Pro)2ONP (L-L-L-D-L) (VIIIb). VIIIb (5.30 mg.) in 5.0 ml. EtOH made up to 10.0 ml. with N NaOH proved to be 92% pure by spectroscopic comparison of its extinction curve at 400 mμ with that of carbobenzoxy-L-leucine p-nitrophenyl ester.

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VIIIb (500 mg.) in 10 ml. CF3CO2H treated slowly with 10 ml. H2O at -5 to 0°, the mixture kept 15 min. at -5° and filtered, the filtrate and aqueous CF3CO2H washings evaporated at -80° in high vacuum (with Dry Ice-Me2CO trap), and the dry residue washed thoroughly with Et2O gave 400 mg. H(Val-p-Tos-Orn-Leu-Phe-Pro)2-ONP.CF3CO2H (L-L-L-D-L) (VIIIc). VIIIc (390 mg.) in 10 ml. HCONMe and 3 drops AcOH stirred 4 hrs. with dropwise addition of 75 ml. pyridine at 55°, the mixture kept 1 hr. at 55°, evaporated in vacuo and the residue refluxed with 1:1 Et2O-petr. ether and Et2O, the in-soluble powder taken up in 1:1:1 iso-PrOH-MeOH-H2O, the solution filtered through strongly basic and strongly acid ion-exchange columns, the filtrate slowly diluted with H2O at 45°, filtered and the residue dried in vacuo gave 170 mg. 80% pure cyclo-(Val-p-Tos-Orn-Leu-Phe-Pro)2.2H2O (L-L-L-D-L)2 (X), chromatographed over neutral Al2O3, the column washed with 9:1 C6H6-CHCl3, eluted with CHCl3 and EtOAc, and the product crystallized from 65% EtOH to give 92.2 mg. prisms, m. 318° (decomposition). **Purification** by Craig countercurrent distribution in 1:1 CCl4-85% MeOH gave pure X, m. 319° (decomposition), [α]D24 -186.3 ± 10° (c 0.669, AcOH). I.2HCl (30 mg.) in 5 ml. pyridine kept 5 hrs. at 40° with 200 mg. p-MeC6H4SO2Cl, the mixture evaporated and the product washed with 1:1 Et2O-petr. ether and H2O, the powdery product chromatographed as above and crystallized from 65% alc. gave X, m. 319° (decomposition), [α]D24 -182.5 ± 10° (c 0.596, AcOH). X (140 mg.) stirred with 80 ml. liquid NH (distilled over Na) and treated portionwise with 70 mg. Na, the permanently-blue solution decolorized with NH4Cl, the solution evaporated in vacuo and the residue taken up in N HCl, filtered and the product recrystd. from alc. N HCl or **purified** by countercurrent distribution in 10:7:3 CHCl3-MeOH-0.01N HCl gave 70-90% cyclo-(Val-Orn-Leu-Phe-Pro)2.2HCl (L-L-L-D-L)2 (Xa), m. 278-9°. The identification of X and Xa with I ditosylate and I.2HCl by analysis, m.p., specific rotation, comparison of infrared spectra and x-ray powder diagram proved the structure of I as a cyclic **decapeptide.** Xa had identical antibiotic acitivity on 19 strains of microorganisms as natural I.

=> dis his

(FILE 'HOME' ENTERED AT 15:35:23 ON 09 DEC 2005)

FILE 'REGISTRY' ENTERED AT 15:36:15 ON 09 DEC 2005
E NONAPEPTIDE/CN 5
E DECAPEPTIDE/CN 5
L1 5 S (METHANOL OR ETHANOL OR PROPANOL OR ISOPROPANOL)/CN
L2 14 S (METHYL ACETATE OR ETHYL ACETATE OR METHYL PROPIONATE OR ETHY
L3 2 S (".TERTIARY.- BUTYL ACETATE" OR ACETIC ACID OR PROPIONIC ACID

FILE 'MEDLINE, BIOSIS, EMBASE, CAPLUS' ENTERED AT 15:39:41 ON 09 DEC 2005
L4 23205 FILE MEDLINE
L5 34113 FILE BIOSIS
L6 39078 FILE EMBASE
L7 417772 FILE CAPLUS
TOTAL FOR ALL FILES
L8 514168 S L2 OR L3 OR METHYL ACETATE OR ETHYL ACETATE OR METHYL PROPION
L9 33366 FILE MEDLINE
L10 46001 FILE BIOSIS
L11 44680 FILE EMBASE
L12 223501 FILE CAPLUS
TOTAL FOR ALL FILES
L13 347548 S ACETIC ACID OR PROPIONIC ACID

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L14        50602 FILE MEDLINE
L15        69093 FILE BIOSIS
L16        64225 FILE EMBASE
L17       544494 FILE CAPLUS
     TOTAL FOR ALL FILES
L18       728414 S L8 OR L13
L19       103185 FILE MEDLINE
L20       172173 FILE BIOSIS
L21       141509 FILE EMBASE
L22       506717 FILE CAPLUS
     TOTAL FOR ALL FILES
L23       923584 S L1 OR METHANOL OR ETHANOL OR PROPANOL OR ISOPROPANOL

     FILE 'CAPLUS' ENTERED AT 15:41:51 ON 09 DEC 2005
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               E E3+ALL
               E DECAPEPTIDE/CT
               E E4+ALL

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L24         3301 FILE MEDLINE
L25         3454 FILE BIOSIS
L26         3129 FILE EMBASE
L27         5580 FILE CAPLUS
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L28        15464 S NONAPEPTIDE? OR DECAPEPTIDE?
L29           11 FILE MEDLINE
L30           17 FILE BIOSIS
L31            9 FILE EMBASE
L32           17 FILE CAPLUS
     TOTAL FOR ALL FILES
L33           54 S L28 AND (DISSOL? SOLVENT? OR (WATER AND L23))
L34            3 FILE MEDLINE
L35            5 FILE BIOSIS
L36            3 FILE EMBASE
L37            5 FILE CAPLUS
     TOTAL FOR ALL FILES
L38           16 S L33 AND L18
L39            8 DUP REM L38 (8 DUPLICATES REMOVED)
L40           15 FILE MEDLINE
L41           12 FILE BIOSIS
L42            8 FILE EMBASE
L43           22 FILE CAPLUS
     TOTAL FOR ALL FILES
L44           57 S PURIF? AND L28 AND (PRECIPIT? SOLVENT OR L18)
L45           13 FILE MEDLINE
L46            9 FILE BIOSIS
L47            6 FILE EMBASE
L48           18 FILE CAPLUS
     TOTAL FOR ALL FILES
L49           46 S L44 NOT L38
L50            0 FILE MEDLINE
L51            0 FILE BIOSIS
L52            0 FILE EMBASE
L53            3 FILE CAPLUS
     TOTAL FOR ALL FILES
L54            3 S L49 AND ISOLAT? AND DRY?
L55           13 FILE MEDLINE
L56            9 FILE BIOSIS
L57            6 FILE EMBASE
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L58             15 FILE CAPLUS
     TOTAL FOR ALL FILES
L59             43 S L49 NOT L54
L60             28 DUP REM L59 (15 DUPLICATES REMOVED)

=> log y
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    88.72     270.27

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)          SINCE FILE      TOTAL
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CA SUBSCRIBER PRICE                                   -12.41     -12.41

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